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Some factors affecting the germicidal efficiency of chloramines

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SOME FACTORS AFFECTING THE GERMICIDAL EFFICIENCY OF CHLORAMINES

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I. INTRODUCTION

At the present time chlorine is quite generally employed as a germicidal agent for producing safe water supplies. Although calcium and sodium hypochlorites were originally used, gaseous chlorine has largely replaced these hypochlorites, but under certain conditions these compounds are still used where chlorine gas can not be conveniently employed.

The ammonia-chlorine process has recently been introduced to avoid odors and tastes sometimes produced by chlorine alone. This process consists essentially of the addition of ammonia either as a salt, the hydroxide, or the gas, prior to chlorination.

Chloramines are formed as a result of the reaction between chlorine and ammonia or other amino compounds. The chemist includes in the term "chloramine", all amino ($-NH_2$) or imino ($=NH$) groups in which the hydrogen has been replaced in whole or in part by chlorine. To those in the medical and pharmaceutical professions the term "chloramine" refers more or less specifically to the mono- and di-chlorine substituted toluine sulphonamide derivatives, usually known as chloramine-T, halazone or dichloramine-T.

Some investigators have pointed out that chloramines are more stable than chlorine and claims for effectively preventing phenolic tastes during chlorination have been made for the

ammonia-chlorine process. Waters in which chloramines are present often carry higher residuals of available chlorine than do waters in which free chlorine is used. The ammonia-chlorine process has been recommended for the following purposes:

1. To prevent certain phenolic tastes developed during chlorination

2. To prevent after-growths

3. To control algae

4. To ensure a high residual without the development of chlorinous tastes and odors

5. To maintain a residual throughout the water system to protect it from pollution subsequent to chlorination, and to prevent after-growths.

Numerous conflicting results concerning the germicidal efficiency of chlorine and chloramines have been reported in recent years. Many of the investigations have been carried out without adequate control of various factors which might influence the results. Tests of germicidal efficiency have been made on hypochlorite solutions with and without additions of ammonia. Other tests have been made on the use of gaseous chlorine in the presence and in the absence of ammonia. Hypochlorites in the presence of sufficient ammonia to form chloramines, may be more alkaline than chlorinated water or the chloramines formed by the addition of ammonia to chlorinated water, yet in many of the experiments reported, the reaction (pH) has been neither controlled nor determined.

Since reaction in certain ranges has been shown to have a marked influence on the germicidal efficiency of hypochlorite and chloramine-T it seems that it might also be important with chloramines.

Little has been known about the chemical nature of many of the waters in which the chlorine and ammonia-chlorine processes have been compared. The presence of organic matter in even very low concentrations may alter the results obtained.

The high losses of residual chlorine which occur when certain chlorine to ammonia ratios are used, and the germicidal efficiencies of solutions with varying chlorine to ammonia ratios have neither been adequately studied nor explained.

Studies of both hypochlorites and chloramine-T have shown that concentration as well as temperature greatly influenced germicidal efficiencies. It was felt that a study to determine the effect of temperature and concentration on the germicidal efficiency of chloramines would be of significance. Since different techniques have been used by different investigators, it is difficult to compare results from various experiments. It was felt that carefully controlled experiments might help to clarify some of the results which appear to be contradictory.

II. HISTORICAL

A. Chloramine as a Germicide

Raschig in 1907 discovered chloramine (NH_2Cl) and three years later the germicidal properties of this compound were first demonstrated by Rideal (1910). This investigator also demonstrated that the addition of ammonia to sodium hypochlorite destroyed the bleaching activity in acid solution. Race (1918) reported the same to be true for ammonia and calcium hypochlorite. He stated that if the bleaching effect of hypochlorites was due to oxidation, the oxidizing power of hypochlorites was destroyed and the property of oxidizing organic matter in water was also destroyed.

The first use of chloramines as a germicide in a public water supply was made by Race (1918). From experiments conducted by this investigator he concluded that the germicidal action of chlorine and chloramines was not due to nascent oxygen. Dakin, Cohen, Daufresne, and Kenyon (1917) came to the same conclusion with respect to hypochlorite in the treatment of wounds. The strong germicidal action was attributed to the formation of chloramines in the wound by the action of hypochlorous acid upon amino acids and proteins. Similar compounds prepared in the laboratory were found to give excellent results in reducing wound infection.

Rideal (1910) assumed that the persistent germicidal action of hypochlorites in sewage was due to the formation of chloramines and chloramine derivatives. He noted that during the chlorination of sewage the first rapid consumption of chlorine was followed by a slower action which continued for days in some cases, and was accompanied by a germicidal action after free chlorine or hypochlorite was no longer present.

B. Relative Germicidal Efficiency of Chlorine and Chloramines

Rideal (1910) using electrolyzed hypochlorite with 1% available chlorine found the carbolic acid coefficient of this germicide to be 2.13. When one equivalent of ammonia was added, the coefficient was increased to 6.36. From the reaction:

$$NH_3 + NaOCl = NH_2Cl + NaOH$$

reported by Rideal it may be seen that one molecule of NH_3 reacts with one molecule of NaOCl. In parts per million this is 70.91 p.p.m. available chlorine reacting with 17.03 p.p.m. NH_3, or a ratio of approximately 4.2:1, (the theoretical ratio required for the production of monochloro-amine). The 1% available chlorine employed by Rideal would be 10,000 p.p.m. and an equivalent of ammonia (from ammonium chloride) would be 2,400 p.p.m. It should be pointed out that a solution of 10,000 p.p.m. available chlorine made from sodium hypochlorite would

have an alkaline reaction.

Race (1918) reported experiments in which ammonia was added to bleach solution and stated that 0.20 p.p.m. available chlorine and 0.10 p.p.m. ammonia were approximately as effective as a germicide as 0.60 p.p.m. available chlorine alone. The water used was Ottawa River water containing 25.0 to 35.0 p.p.m. alkalinity, traces of ammonia and organic matter. It should be noted that the concentration of 0.10 p.p.m. ammonia was added ammonia and did not include traces which were present in the river water before the experiment was started. It seems likely that these traces of ammonia might markedly influence the results obtained.

Tilley (1920) set up some experiments designed to verify the findings of Race (1918) and Rideal (1910) concerning the greater germicidal efficiency of an electrolytic hypochlorite solution upon the addition of ammonia. For this study he used both Dakin's solution* and chlorinated water. His data showed that Dakin's solution plus an equivalent# amount of ammonia had a greater germicidal power than Dakin's solution without the ammonia. Chlorine water plus an equivalent amount of ammonia showed a decrease in germicidal power over chlorine water alone. A slightly modified Rideal-Walker method was used for this study.

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* The term "Dakin's solution" as used by Tilley signifies a neutral solution of sodium hypochlorite.

\# One molecule of ammonia was added for each molecule of sodium hypochlorite of the Dakin's solution.

Harold and Ward (1924) studying the germicidal efficiency of chlorine and chloramines, using natural waters containing collodial organic matter and ammonia reported that "chlorine gas gives better results than the same quantity of chlorine as bleaching powder solution; further that with bleaching powder the addition of an equal amount of ammonia tends to improve slightly the killing power; but with twice the quantity of ammonia the result is worse than with bleaching powder alone. With chlorine gas the addition of an equal quantity of ammonia causes a definite increase in the killing power and the addition of further ammonia tends to nullify this."

In all probability the reaction (pH) of a bleaching powder solution is more alkaline than is that of a chlorine gas solution. Differences in reaction (pH) of the two solutions may explain the differences in germicidal efficiency.

An equal quantity of ammonia as used by Harold and Ward is a ratio of available chlorine to ammonia of 4.2:1. It should be pointed out, however, that the natural waters used contained ammonia so that the ratio of available chlorine to ammonia in the test solution would be somewhat less than 4.2:1, depending upon the amount of ammonia present in the water before the experiment was started.

Wade, Archibald and Whittaker (1928) concluded that calcium hypochlorite solution containing 500 p.p.m. available chlorine can not be depended upon for the destruction of human tubercle bacilli. However, the use of chloramine in strengths

of 93 to 95 p.p.m. available chlorine for a period of three minutes was effective. Stronger solutions of chloramine applied for a shorter time were also effective.

Molwerda (1928) and Gerstein (1931) studying the germicidal efficiency of chlorinated water with and without additions of ammonia reported results showing that chlorinated water is a more efficient germicide when ammonia is not present.

Tilley and Chapin (1930) studied the germicidal action of chlorine and chloramines against Bacillus anthracis spores. They were interested in finding the concentrations and times required for chlorine and chloramines to kill anthrax spores present in tannery effluents. Germicidal solutions were prepared and the anthrax spores added. Subcultures were made into tubes of beef infusion both at appropriate time intervals. All experiments were carried out at room temperature. The table below has been taken from the report by these investigators.

Disinfectant	Available Chlorine p.p.m.	Exposure Time in Minutes					
		15	30	45	60	90	120
Nitrogen Trichloride	4	-	-	-	-	-	-
Chlorine	4	+	+	+	+	+	+

From the above table it may be seen that with 4 p.p.m. available chlorine as nitrogen trichloride no growth was obtained by subculturing after 15 minutes, while with chlorine, using the

same concentration of available chlorine, subcultures showed growth after two hours. No reactions (pH) were stated for these two disinfectant solutions and the reactions (pH) may not be the same.

Charlton and Levine (1937), comparing the germicidal efficiency of hypochlorite and the chloramine formed from the reaction of hypochlorite and ammonia, both at pH 9.0 in unbuffered solutions, concluded that at this reaction (pH) the germicidal efficiency of chloramine was as great or greater than that of hypochlorite.

Spector, Baylis and Gallans (1934) studying the germicidal action of chlorine and chloramine against cysts of both Endamoeba histolytica and Endamoeba coli, in water between pH 8.0 and 8.5 concluded that much larger quantities of chloramine than chlorine are required to kill the cysts, indicating that chlorine is more effective.

McDonnell (1938) compared chlorine and chloramines for sterilizing water supplies containing spore-forming organisms. The test organism used was Bacillus subtilis. Employing approximately 20,000 spores per ml., his results indicated that 30 p.p.m. chlorine had no appreciable effect upon the bacterial population after exposure for a period of one hour. He concluded that chlorine even in concentrations of 30 p.p.m. was of no value in the disinfection of water supplies containing spore-forming organisms. This investigator then studied the effect of the ammonia-chlorine process (chloramine), in which

the chlorination was preceded by ammoniation. His findings show that a chloramine dosage of 10 p.p.m. ammonia followed by 30 p.p.m. chlorine yielded a sterile water in less than one half hour contact. The reaction (pH) was not stated and the residual chlorine showed a constant drop of 30% over a period of six hours.

C. Factors Affecting Germicidal Action of Chlorine* and Chloramines

1. Reaction (pH)

Holwerda (1928), using "B. coli", studied the influence of reaction (pH) on the bactericidal efficiency of chloramine using 0.5 p.p.m. available chlorine. The table presented below is taken from the report of Holwerda.

Showing Effect of Reaction (pH) on the Germicidal Efficiency of Chloramine (NH_2Cl)
(0.5 p.p.m. available chlorine; 27° C.)

Time of Exposure (in min.)	pH 4.5	pH 6.8	pH 8.5
20	-	+	+
30	-	-	+
40	-	-	+
50	-	-	+
60	-	-	-

(+ signifies growth; -, no growth)

*Charlton (1933) and Rudolph (1938) have given comprehensive historical reviews of the factors affecting the germicidal action of chlorine and hypochlorites. In order to avoid repetition the following historical review will be confined to chloramines.

From the above table it may be seen that at pH 4.5 a subculture into lactose broth showed no growth at the end of a 20 minute period of exposure. When the acidity was lowered to pH 6.8 the killing time was increased to between 20 and 30 minutes and at pH 8.5 the killing time was still longer, namely between 50 and 60 minutes. The chlorine residual was still 0.5 p.p.m. as estimated by the O-tolidine method, hence the lesser disinfection with more alkaline solutions was not due to decomposition of NH_2Cl. Similar results to these indicated above were obtained when 0.5 p.p.m. chlorine as NH_2Cl was compared at pH 4.0, 6.8 and 8.6, that is, an increase in the killing time as the reaction becomes more alkaline.

The table presented below has been taken from the report of Tilley and Chapin (1930).

Bactericidal Efficiency of Monochloro-amine and Dichloro-amine Against the Spores of *B. anthracis*

Disinfectant	Available Chlorine (p.p.m.)	Exposure Time in Minutes					
		15	30	45	60	90	120
Monochloro-amine pH 9.0	20	+	+	+	+	-	-
	40	+	+	+	-	-	-
	60	+	+	-	-	-	-
	80	+	+	-	-	-	-
Dichloro-amine pH 4.8	20	+	+	+	+	-	-
	40	+	+	-	-	-	-
	60	+	+	-	-	-	-
	80	+	-	-	-	-	-

(+ signifies growth; -, no growth)

It will be noted in the above table that there is no difference in the killing times for 20 p.p.m. available chlorine as monochloro-amine at pH 9.0 and as dichloro-amine at pH 4.8. The killing times were determined by subculturing into beef infusion broth. When the concentration of available chlorine was increased to 80 p.p.m., it may be seen that a shorter killing time was obtained at the lower pH, namely, a killing time between 30 and 45 minutes at pH 9.0 compared to a killing time between 15 and 30 minutes at pH 4.8.

Charlton and Levine (1937) carried out some experiments to determine the effect of reaction (pH) on the germicidal efficiency of chloramine-T at 25° C. The test organism used was Bacillus metions (spores) and three concentrations of the germicide were employed, namely, 1,000, 2,000 and 4,000 p.p.m. available chlorine. Their results show that as the reaction (pH) becomes more alkaline the killing time (time to kill 99 percent of the exposed spores) becomes longer. For example, using 4,000 p.p.m. available chlorine at pH 6.0, 6.2, 6.8, 7.3 and 8.8 the killing times were approximately 2.5, 5, 13, 20 and 23 hours respectively.

2. Concentration

Tilley and Chapin (1930) studied the effect of concentration of chloramines using Bacillus anthracis spores, (100,000 per ml.) in the test solution. They employed buffered solutions at room temperature and subcultured into

beef infusion broth after various periods of exposure. The table below was taken from the report of Tilley and Chapin (1930).

Bactericidal Efficiency of Monochloro-amine, and Nitrogen Trichloride Against the Spores of B. anthracis

Disinfectant	Available Chlorine (p.p.m.)	Exposure Time in Minutes					
		15	30	45	60	90	120
Monochloro-amine pH 9.0	20	+	+	+	+	-	-
	40	+	+	+	-	-	-
	60	+	+	-	-	-	-
	80	+	+	-	-	-	-
Dichloro-amine pH 4.8	20	+	+	+	+	-	-
	40	+	+	-	-	-	-
	60	+	+	-	-	-	-
	80	+	-	-	-	-	-
Nitrogen Trichloride (pH not reported)	2	+	+	+	+	+	+
	4	-	-	-	-	-	-

(+ signifies growth; -, no growth)

From the above table it may be seen that at pH 9.0 (using monochloro-amine) with 20 p.p.m. available chlorine the killing time was between 60 and 90 minutes. When the concentration was doubled (40 p.p.m. available chlorine) the killing time was decreased to between 45 and 60 minutes. By further increasing the concentration of monochloro-amine to 60 p.p.m. available chlorine the killing time was still further decreased

to between 30 and 45 minutes. Increasing the concentration of the germicide to 80 p.p.m. available chlorine did not decrease the killing time below that obtained with 60 p.p.m. available chlorine.

The same general tendency toward a much shorter killing time with a higher concentration of the germicide was obtained at pH 4.8 (using dichloro-amine) as can readily be seen from the above table. For example, with 20 p.p.m. available chlorine the time required to kill the B. anthracis spores was between 60 and 90 minutes and when the concentration of available chlorine was increased to 80 p.p.m. this time to kill the spores was shortened to between 15 and 30 minutes.

Also, from the above table it may be seen that by increasing the concentration of nitrogen trichloride from 2 p.p.m. available chlorine to 4 p.p.m. the killing time was decreased from more than 120 minutes to less than 15 minutes.

Charlton and Levine (1937) studied the effect of concentration on the germicidal efficiency of chloramine-T (at 25° C.) using Bacillus anthracis spores. They found that by doubling the concentration of the germicide, the killing time (time to kill 99 percent of the exposed spores) was reduced by approximately 50 percent. For example, at pH 6.2 the killing times were 27, 12 and 5.4 hours for the 1,000, 2,000, and 4,000 p.p.m. concentrations of available chlorine, respectively.

McDonnell (1938) made some studies which show the effect of concentration on the germicidal efficiency of chloramines. A suspension of 20,000 Bacillus subtilis spores was exposed to different concentrations of chloramine prepared in water taken from a city water supply. It should be pointed out that traces of organic matter and ammonia were very likely present and these compounds probably have influenced the results obtained. The test solutions were approximately neutral in reaction (pH). By increasing the concentration of the germicide the time required to sterilize the solution was markedly diminished. For example, 3-1/3 p.p.m. ammonia and 10 p.p.m. chlorine sterilized the test solution in a time interval of between one and two hours. By doubling these concentrations the killing time dropped to less than one-half hour and by trebling the concentration, that is, increased to 10 p.p.m. ammonia and 30 p.p.m. chlorine, the test solution was sterilized immediately.

3. Temperature

Holwerda (1928) made some investigations to show the effect of temperature on the germicidal efficiency of chloramine (NH_2Cl) against "B. coli". Employing 0.5 p.p.m. chlorine as NH_2Cl at 15° C., reaction (pH) near neutrality, and starting with approximately 200 "B. coli" cells per ml. he found that the bacterial count had dropped very

little in 60 minutes. However, when the same study was made at 27.5° C. the bacterial count was reduced to almost zero at the end of 60 minutes. He concluded that a 60 minute exposure at 15° C. is comparable to a 20 minute exposure at 27.5° C.

Gerstein (1931), studying the effect of temperature on the germicidal efficiency of chloramines, concluded that lowering the temperature of the water had a decided effect in reducing the bactericidal efficiency of the ammonia-chlorine treatment. He stated that the temperature range was controlled between the limits 0° to 20° C., which represents the normal temperature variation in the Chicago water supply. After a 5-minute contact there was very little difference in bactericidal effect due to temperature. However, after 30 minutes contact the higher temperature showed a greater efficiency, and after a 2-hour contact the increased efficiency due to the higher temperature was quite pronounced.

Charlton and Levine (1937) made studies to determine the effect of temperature on the germicidal efficiency of chloramine-T. A suspension of Bacillus metiens spores was employed. They found a marked effect of temperature on the killing time for solutions containing 2,000 p.p.m. available chlorine. For a rise of 10° C. the killing time was reduced 82 percent for an initial reaction of pH 6.0 and 71.5 percent for an initial reaction of pH 8.7.

4. Organic matter

Tilley (1920) investigated the germicidal efficiency of Dakin's solution in the presence of ammonia alone and in the presence of ammonia plus horse serum. His results indicate that the addition of a molecular equivalent of ammonia to Dakin's solution not only greatly increased the germicidal value against "Bacillus typhosus" but to a large extent prevented depreciation of germicidal value on addition of blood serum.

Similar studies were made using chlorine water instead of Dakin's solution. The following table, showing data obtained when employing chlorine water, is from the report of Tilley (1920).

Effect of Ammonia upon the Germicidal Activity of Chlorine in Aqueous Solution against "Bacillus typhosus"

Concentration of Chlorine	Exposure Time in Minutes					
	$2\frac{1}{2}$	5	$7\frac{1}{2}$	10	$12\frac{1}{2}$	15
Without ammonia						
1 to 12,000	-	-	-	-	-	-
With ammonia*						
1 to 12,000	+	+	+	-	-	-
Without ammonia; 10% blood serum added						
1 to 2,000	+	+	+	+	+	+
With ammonia;* 10% blood serum added						
1 to 2,000	-	-	-	-	-	-

(+ signifies growth; -, no growth)
* one molecular equivalent of ammonia

It will be noted in the above table that the addition of ammonia to chlorinated water decreased, rather than increased, the germicidal value of the chlorine in the absence of organic matter (blood serum), but the ammonia did tend to prevent depreciation of germicidal activity upon the addition of blood serum. For example, in the absence of ammonia (and no organic matter), a chlorine concentration of 1 to 12,000 killed "Bacillus typhosus" in less than $2\frac{1}{2}$ minutes. When a molecular equivalent of ammonia was added however, the same concentration of chlorine required between $7\frac{1}{2}$ and 10 minutes to kill the test organism. In the presence of 10 percent blood serum (without ammonia) a chlorine concentration of 1-2000 did not kill the test organism in 15 minutes. When a molecular equivalent of ammonia was added to a solution of the same concentration of chlorine with blood serum, however, the test organism was killed in less than $2\frac{1}{2}$ minutes. Studies of a like nature were also carried out using Bacillus anthracis instead of "Bacillus typhosus", and results were obtained corresponding to those reported above.

Harold and Ward (1924) reported results from extensive experiments in which they studied the effect of additions of organic matter (broth cultures) on the germicidal efficiency of chlorine solutions. They stated that by reducing the amount of broth culture (added to chlorine solution) from 0.5 cc. to 0.25 cc., the residual chlorine was lower (after 45 minutes), yet a greater killing power was obtained. However, the tables reported show that the chlorine concentration (added) as well as the concentration of broth culture was varied. For example, when

0.5 cc. of the broth culture was used, the chlorine concentration added was 5 p.p.m. and when the concentration of broth employed was lowered to 0.25 cc., the chlorine added was reduced as well to 2 p.p.m. Since two variables are involved in the experiments reported by these investigators it is difficult to draw any definite conclusions regarding the effect of organic matter on the germicidal efficiency of chlorine.

Holwerda (1930) stated that when hypochlorite acts on organic compounds such as proteins or their decomposition products, reaction products can be formed in which the chlorine atom is fixed to the nitrogen atom. These products, which may still give a color reaction with O-tolidine, can, to a certain degree, still exercise a disinfecting actio , but it is a question whether they may be placed in the same class as NH_2Cl when their properties as germicides are considered.

Guiteras and Schmelkes (1934) studying the loss of efficiency of chlorine in the presence of organic matter concluded that this loss in efficiency is due to the reaction of the hypochlorites with the alpha-amino acids to form chloramino acids which break down to aldehydes or ketones, ammonia, carbonic acid, and sodium chloride. With aromatic or heterocyclic amino acids, partial chlorination of the ring takes place in addition to oxidation. Chlorine which has been reduced in oxidizing or chlorinating a ring is of no value as a germicide. They concluded that any chlorine which has substituted a hydrogen atom of the amino group and which is present as $-N=Cl_2$ or $=N-Cl$ is

still an active germicide.

According to McCulloch (1936) the chloramines are less affected than chlorine by the presence of organic matter. Chlorine is ordinarily considered unstable in the presence of large amounts of organic matter and is consequently usually not recommended for a germicide under such conditions. He stated that Salmonella pullorum in the presence of a 5 percent suspension of chicken manure was killed using a hypochlorite solution of 130 p.p.m. available chlorine. A definite lag period preceded the maximum germicidal effect and it was suggested that possibly chloramines were formed by the action of the hypochlorite upon the ammonia present in the chicken manure. McCulloch also reported similar results using E. typhi and human feces.

Tilley and Chapin (1930) studying the disinfection of tannery effluents using Bacillus anthracis spores concluded that effluents sufficiently chlorinated to become anthrax-free in less than two hours can receive much additional chlorine without showing evidence of "saturation". They explain that a large amount of the added chlorine does not appear as available chlorine. Apparently some new compounds are formed which are sufficiently germicidal to kill the resistant spores but which do not give the usual tests for available chlorine. Such mixtures do not bleach certain indicator solutions nearly so rapidly as do very small concentrations of primary chlorine, namely, chlorine in the

form of hypochlorous acid or hypochlorites. It is supposed that a N-chloro compound is produced as a result of the reaction between chlorine and the nitrogenous substances present in the effluent.

5. Ratio of chlorine to ammonia

The ratio of chlorine to ammonia in a solution has been shown by a number of investigators to be an important factor in determining germicidal efficiency and in maintaining a chlorine residual. Amino-acids and other compounds having amino groups have likewise been shown to be important in these respects.

Dakin (1916) has shown that if increasing quantities of blood serum are added to a constant volume of hypochlorite solution there is a resulting reduction of available chlorine but this reduction does not take place quantitatively. Large quantities of serum may destroy less hypochlorite than small quantities.

Wright (1926) investigated the reactions which take place between sodium hypochlorite and milk. With the addition of small quantities of milk he reported that available chlorine fell to about one-third of its initial value. However, with larger quantities of the protein, the destruction of chlorine was less. Other proteins and amino acids were likewise studied. Wright explained this phenomenon by stating that

probably sodium hypochlorite acts either as an oxidizing or as a chlorinating agent. He stated further that there is a possibility of variable stability of the chloramino-derivatives formed by this reaction. The nature of the reaction was considered to depend on the relative proportions of chlorine and amino-acid present; with excess chlorine it was regarded as oxidation and with excess amino-acid, as chlorination.

Goresline (1928) demonstrated that if different initial concentrations of chlorine were added to a sample of milk, there was more chlorine absorbed by the sample containing the highest concentration of chlorine. This increased chlorine absorption was not in proportion to the amount of chlorine added. For example, with a constant milk concentration of 2 percent the additions of 25, 50 and 100 p.p.m. chlorine showed chlorine demands of approximately 18, 22 and 26 p.p.m. respectively. Similar results were obtained with the addition of chlorine to peptone.

Race (1918) carried out some experiments with a view to determining the most desirable ratios (by weight) of chlorine to ammonia for germicides and found that ratios between 8:1 and 1:2 showed approximately the same germicidal velocity. He stated that the action of the ammonia on the oxidizing power of bleach, as measured by the indigo test, was found to be disproportionate to the amount of ammonia added. The following table has been taken from the report of Race (1918).

Ratio of Available Chlorine to Ammonia (by weight) Added to Ottawa River Water	Percent of Original Concentration of Chlorine Recovered after 20 hours
Infinity (ammonia absent)	25.1
8:1	67.3
4:1	88.5
2.7:1	92.8
2:1	96.2

It should be noted in the table presented above that Ottawa River water was used for these studies. Since the ammonia concentration of this water was not determined, it is probable that the ratios of chlorine to ammonia may be lower than those stated, depending upon the amount of ammonia present in the water before the experiment was started. The percent of the original chlorine concentration recovered after 20 hours was very different when different ratios of available chlorine to ammonia were added and this percent increased as the ratio of available chlorine to ammonia decreased.

It can be seen then, that residual chlorine in a solution containing ammonia is dependent, to a certain extent, upon the ratio of chlorine to ammonia.

Due to the high cost of bleach during the World War (1914-18) Race (1918) determined the relative germicidal efficiencies of bleach (calcium hypochlorite) alone and bleach plus ammonia

when used on a large scale for the purification of water. He found that the ammonia-bleach process was effective in lower concentrations than bleach alone and was consequently less expensive. It was stated that previous experiences had shown that although a dosage of approximately 1.5 p.p.m. available chlorine was required to reduce the "B. coli" index to 2.0 per 100 cc., it was also possible to reduce the chlorine dosage to 0.25 p.p.m. with 0.06 p.p.m. ammonia (a ratio of approximately 4.2:1) without adversely affecting the bacteriological purity of the supply at the tap. The times required for these reductions in the "B. coli" index were not reported although it was stated that all experiments were carried out under similar physical and bacteriological conditions. The lowest ratio of available chlorine to ammonia used during tests conducted by Race was 4.2:1, which is approximately the theoretical ratio required for the production of monochloro-amine.

Tilley (1920) reported that the optimum amount of ammonia to be used with hypochlorite as a germicide was found to be approximately one-half the molecular equivalent in the absence of organic matter (blood serum). With excess ammonia (two molecular equivalents) the germicidal action was diminished rather markedly.

Harold, (1925) working with natural waters, reported that the method of adding chlorine and ammonia to water had

an important influence upon the germicidal efficiency of the resulting solution. The ratio of chlorine to ammonia was likewise considered important. He stated that, " two compounds are formed endowed with considerable germicidal powers: (1) by a combination of equivalents of chlorine and ammonia; (2) by interaction of two equivalents of chlorine with one of ammonia and that the former can be changed to the latter by the addition of an equivalent of chlorine. If further additions of chlorine are made, compounds containing higher ratios of chlorine are not formed under these conditions, and the chlorine remains as free chlorine" .

The fact that certain municipal water supplies may contain ammonia in varying quantities and that these water supplies are chlorinated, led Holwerda (1930) to make some chemical studies of solutions containing various ratios of chlorine to ammonia.

The water used for these experiments contained very little organic matter and no ammonia. The possibility that traces of organic matter present might influence the final chlorine residual should not be overlooked. The reaction (pH) of the water was near neutrality. In the following table, the percentages of chlorine remaining after thirty minutes have been calculated from the data reported by Holwerda.

Ratio by Weight of Chlorine to Ammonia (added)	Percent of Added Chlorine Recovered after Thirty Minutes
5.0	70
5.7	33
6.3	14
7.5	17
8.4	27
10	33
12.6	46

It will be noted from the above table that the percent of residual chlorine remaining after thirty minutes is quite different when different ratios of chlorine to ammonia are added. As the ratio of chlorine to ammonia increases, the percent of added chlorine recovered after thirty minutes, drops to a minimum, and this minimum is followed by a rise. For example, with a ratio of 5.0, 70 percent of the added chlorine remained after thirty minutes. When this ratio was increased to 6.3, only 14 percent of the added chlorine remained while with a still higher ratio (12.6), the percent of residual chlorine again rose to a higher value, namely to 46 percent.

Gerstein (1931) reported that increasing the ratio of chlorine to ammonia hastens the bactericidal effect. He states

that when the chlorine dosage is progressively increased in the presence of a small amount of ammonia, a point is reached at which the residual chlorine breaks down and disappears more rapidly than if a lower dosage of chlorine is used. It has been pointed out by this investigator that the same residual chlorine may be obtained by using different ratios of ammonia and chlorine, yet these various combinations may have widely varying bactericidal efficiencies.

D. Some Chemical Properties of Chloramines

Raschig (1907) produced chloramine by the interaction between dilute solutions of sodium hypochlorite and ammonia according to the reaction:

$$NaOCl + NH_3 = NH_2Cl + NaOH.$$

Bray and Dowell (1917) reported that nitrogen trichloride is formed very rapidly and almost quantitatively according to the following reactions when excess of strong acid is present:

$$NH_3 + 3Cl_2 = NCl_3 + 3H^+ + 3Cl^-$$

$$NH_4^+ + 3Cl_2 = NCl_3 + 4H^+ + 3Cl^-$$

$$NH_4^+ + Cl^- + 3HClO = NCl_3 + H^+ + Cl + 3H_2O.$$

When excess of base is present nitrogen gas is evolved rapidly and almost quantitatively according to the reactions given below:

$$2NH_3 + 3Cl_2 = N_2 + 6H^+ + 6Cl^-$$

$$8NH_3 + 3Cl_2 = N_2 + 6NH_4 + 6Cl^-$$

$$2NH_3 + 3ClO^- = N_2 + 3Cl^- + 3H_2O.$$

The first two equations represent the same reaction except that neutralization of the acid by excess ammonia is shown in the second equation.

Noyes (1917), Noyes and Haw (1920) and Noyes (1920) have also studied the chemical reactions between chlorine and ammonia. Reactions reported by these investigators are quite similar to those reported by Raschig (1907), Bray and Dowell (1917) and others.

Chapin (1929) stated that in solutions more alkaline than slightly above pH 8.5 nothing but monochloro-amine was found; below pH 4.4 nitrogen trichloride was produced with only a trace of chloramine; between pH 4.4 and 8.5 graduated mixtures of the two chloramines resulted.

Chapin (1931) pointed out that the action of excess hypochlorite upon ammonia is known to progress to the formation of nitrogen trichloride. "Stoppage of the chlorination at the stage of either chloro-amine demands first a limited proportion of the chlorinating agent and second a sufficiently high pH." He stated that two fundamental general hypotheses are adequate to account for the various products of decompositions which result from the chlorination of the ammonium ion over a wide range of conditions: Hypothesis I. Under the influence of hydrogen ion, all three chloro derivatives of ammonia yield ammonium ion and hypochlorous acid. Hypothesis II. Under the influence of hydroxyl ion, all three chloro derivatives, of ammonia yield chloride ion, the consequent oxidation

of the residual atoms leading to a variety of associated products.

Berliner (1931) has given a comprehensive discussion of the chemistry of chloramines. According to this investigator "a complete separate chemistry could be written for these compounds in various degrees of concentration". Many of the reactions reported by this investigator and others, namely, Bray and Dowell (1917), Noyes (1917), Noyes and Haw (1920), Noyes (1920), Chapin (1929) and Chapin (1931), probably apply to concentrated solutions only, that is, solutions containing many times the concentrations ordinarily employed as germicides. Few chemical studies have been made on dilute solutions due probably to lack of methods sufficiently accurate for low concentrations.

A number of chemical reactions which chloramines may undergo under certain conditions have been reported by Raschig (1907), Bray and Dowell (1917), Noyes (1917, 1920), Berliner (1931), Chapin (1929, 1931) and others. Holwerda (1930) has summarized some of these. The following equations show reactions of chloramines which may take place under certain conditions:

(1) $3HOCl + 2NH_3 = N_2 + 3HCl + 3H_2O$

(2) $HOCl + 2NH_2Cl = N_2 + 3HCl + H_2O$

(3) $NCl_3 + NH_3 = N_2 + 3HCl$

(4) $NCl_3 + 3NH_2Cl = 2N_2 + 6HCl$

(5) $3NHCl_2 + NH_3 = 2N_2 + 6HCl$

(6) $NHCl_2 + NH_2Cl = N_2 + 3HCl$

$$\begin{array}{l} 2NCl_3 = N_2 + 3Cl_2 \\ \underline{3Cl_2 + 3H_2O = 3HCl + 3HOCl} \end{array}$$

(7) $2NCl_3 + 3H_2O = N_2 + 5HCl + 3HOCl$

$$\begin{array}{l} 2NHCl_2 = N_2 + 2HCl + Cl_2 \\ \underline{Cl_2 + H_2O = HCl + HOCl} \end{array}$$

(8) $2NHCl_2 + H_2O = N_2 + HCl + HOCl$

(9) $3NH_2Cl = N_2 + NH_4Cl + 2HCl$

(10) $NCl_3 + H_2O = NHCl_2 + NOCl$

(11) $NCl_3 + 2H_2O = NH_2Cl + 2HOCl$

(12) $NCl_3 + 3H_2O = NH_3 + 3HOCl$

(13) $NCl_3 + NH_2Cl = 2NHCl_2$

(14) $NHCl_2 + H_2O = NH_2Cl + HOCl$

(15) $NHCl_2 + 2H_2O = NH_3 + 2HOCl$

(16) $NH_2Cl + H_2O = NH_3 + HOCl$

In the reactions (1) to (9) available chlorine and ammonia disappear in a ratio of $3Cl_2$ to $2NH_3$. This is a relation of 6.3 to 1 by weight.

The theoretical ratio of available chlorine to ammonia required for the complete oxidation of the latter may be seen from the following equations:

I. $Cl_2 + H_2O = HOCl + HCl$

II. $HOCl = HCl + O$

III. $2NH_3 + 3O = N_2 + 3H_2O$

Equation I shows that Cl_2 yields one molecule of HOCl and in equation II it may be seen that one molecule of HOCl

yields one atom of oxygen. Upon this basis $3Cl_2$ would yield three oxygen atoms (equations I and II). Two molecules of NH_3 require three oxygen atoms for complete oxidation (equation III), and it becomes evident that chlorine oxidizes ammonia in the theoretical ratio of $3Cl_2$ to $2NH_3$. Since the molecular weight of Cl_2 is 70.92 and that of NH_3 is 17.03 we find a relationship by weight of 212.76 to 34.06 (equal to 6.3 to 1) for the ratio of $3Cl_2$ to $2NH_3$. The actual chlorine added (see equation I) is equal to the "available chlorine" and this ratio of 6.3 to 1 is the ratio of available chlorine to ammonia which is required for the complete oxidation of the latter.

Norman (1936) reported that glycine was rapidly oxidized by hypochlorite. It was stated that at least five times as much chlorine as glycine must be present for completion of the reaction. When such an oxidation takes place, 1 mg. of glycine uses 4.26 mg. of chlorine. Results were likewise reported showing that 6.75 mg. of available chlorine were required for the oxidation of 1 mg. NH_3 (the theoretical ratio is 6.3 to 1). Norman stated that the additional utilization over and above the theoretical value was believed to be due to the formation of a small amount of nitrite. Positive tests for nitrite were obtained in certain cases.

Holwerda (1930) was able to prepare ammonia-free water by adding excess chlorine and allowing the solution to stand

in direct sunlight. From the report of Norman (1936) it would seem that if a ratio of chlorine to ammonia was sufficiently high, ammonia-free water would be formed, due to the oxidation of any ammonia present.

E. Résumé

The historical review presented above shows a lack of agreement of results reported by different investigators, especially regarding the relative germicidal efficiencies of chlorine and chloramines. The lack of control of factors influencing chlorine and chloramine disinfection may explain why such conflicting results have been reported.

III. EXPERIMENTAL

A. Objectives

The objectives of the following experiments were to determine the effect of ammonia on the germicidal properties of chlorine and the influence of reaction (pH), concentration and temperature on the germicidal efficiency of chlorine and chlorine-ammonia mixtures.

B. Methods

1. Test solutions

All water employed in these studies was prepared by redistilling distilled water from an alkaline potassium permanganate solution using a glass still, and was ammonia-free when tested with Nessler's reagent.

Chlorine solutions were prepared by diluting a saturated stock solution made by passing gaseous chlorine into ammonia-free water. This gaseous chlorine was secured from the Chemistry Department. The saturated chlorine solution was held in a glass stoppered bottle painted black on the outside to protect it from light and stored in a refrigerator at approximately 10° C.

The source of ammonia was ammonium sulfate and all calculations were made as NH_3. At the beginning of these experiments, duplicate Kjeldahl ammonia nitrogen determinations were made on the ammonium sulfate and the results showed that the theoretical amount of NH_3 was present in the salt. A stock ammonium sulfate solution was prepared on the day previous to its use by dissolving the required amount of ammonium sulfate in sterile water. The solutions were stored overnight in a refrigerator at about 10° C.

There may be some question as to whether the form in which ammonia is added to a chlorine solution affects the persistence of residual chlorine. Gerstein (1931) made a number of tests to determine the importance of the ammonia source. Ammonium chloride, ammonium sulfate, ammonium carbonate, ammonium alum and ammonium hydroxide showed practically no difference in residual chlorine after a 24 hour period of contact.

All experiments were carried out at controlled and constant reactions (pH) which were maintained by $\frac{N}{20}$ buffer solutions prepared as described in the appendix.

2. Determination of available chlorine.

All chlorine concentrations are expressed as "available chlorine."* Charlton (1933) and McCulloch (1936) have given

* Standard Methods of Water Analysis (p. 231, 1936). One ml. $\frac{N}{100}$ sodium thiosulfate is equivalent to 0.3546 milligrams available chlorine.

excellent reviews of the definitions of the various terms used for expressing chlorine concentrations. Chlorine concentrations were determined by titration in the presence of excess potassium iodide in an acidified solution (HCl) using approximately $\frac{N}{100}$ sodium thiosulfate. The solution of sodium thiosulfate was standardized in acid solution against $\frac{N}{100}$ potassium dichromate, each day that it was used. The reactions involved are as follows:

$$Cl_2 + H_2O = HCl + HOCl$$

$$HOCl + 2HI = H_2O + HCl + I_2$$

$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI$$
(in neutral or acid solution)

$$K_2Cr_2C_7 + 6KI + 14HCl = 8KCl + 2CrCl_3 + 7H_2O + 3I_2$$

The question may arise as to whether ammonia interferes with the determination of "available chlorine". Tilley and Chapin (1930) have found that "when dilute solutions of those chloro derivatives (amino compounds) are assayed by treatment with acidified potassium iodide and subsequent titration with sodium thiosulphate, the mono- and dichloro products, like hypochlorites, afford 'available chlorine' in twice the proportion of the constituent chlorine, as in the equations

$$NH_2Cl + 2HI = NH_4Cl + 2I$$
$$NHCl_2 + 4HI = NH_4Cl + HCl + 4I.$$

Nitrogen trichloride should behave similarly but interference by a side reaction cuts the yield to about 80 per cent

of the theoretical''.

3. Determination of reaction.

Since chlorine solutions are strong oxidizing agents, and the methods formerly generally employed for determining pH (hydrogen, quinhydrone, metal-oxide electrodes and calorimetric methods) are not satisfactory in the presence of such agents, the glass electrode (Coleman model 3D) was used for all pH determinations. The error in measuring pH was not greater than ± 0.05. According to Dole (1937), ''Glass electrode potentials depend only on the concentration of the hydrogen ion and are absolutely unaffected by the presence of other ions, by oxidizing or reducing agents, gases, dissolved organic compounds, colloids or suspended matter. This is strictly true except in the case of certain ions at high pH values and in the case of non-aqueous solvents''. The glass electrode is accurate for pH measurement below about pH 10 but above this value there is an error due to various cations such as potassium, sodium lithium and barium present. For this reason alkaline buffer solutions containing approximately the same concentration of cations as the buffers used in these studies were prepared and standardized by means of a hydrogen electrode. These buffers were then used to calibrate the glass electrode in the alkaline range. No correction for pH was necessary at pH 10, but at pH 11 and above, there was an appreciable alkali error. No studies were made above pH 10,

hence no corrections for alkali error were necessary. The standard reference buffer used throughout this study was $\frac{M}{20}$ potassium acid phthalate (pH 3.97).*

4. Test organism

Bacillus metiens, a description of which has been given by Charlton and Levine (1937), was the test organism used. The organism was found to conform in all of the characteristics described in the reference cited. It was originally isolated from a sample of ginger ale, was first described by Levine, Buchanan and Lease (1927) and named Bacillus metiens by Charlton and Levine (1935, 1937) who listed the following advantages of using spores in studies of chlorine disinfection: "Higher concentrations of the disinfectant could be employed; studies over wider ranges of temperature and reaction (pH) were possible, suspensions of the test organism in almost the same numbers and of practically uniform resistance could be readily made up from day to day; the spores did not exhibit appreciable changes in numbers of viable cells or in resistance to chloramine-T after exposures in distilled water for times longer than were employed in the test experiments". This organism (Bacillus metiens) is particularly suitable for studies of germicides since it grows well on ordinary laboratory media and forms distinct, discrete colonies on nutrient

* Clark, Determination of Hydrogen Ions, p. 485, (1928).

agar at 30° C. within 24 hours as has been pointed out by Levine, Buchanan and Lease (1927).

Spore suspensions were prepared by growing the organism on nutrient agar (Difco) at 30° C. for 20 days. At the end of this time the spores were washed from the slants using Butterfield's* formula C water, filtered through Whatman No. 2 filter paper to remove clumps and heated at 80° C. for 10 minutes to kill vegetative cells. This method for preparation of spores was first used by Rudolph and Levine (1938). They reported that these suspensions showed no appreciable change in resistance to a commercial calcium hypochlorite solution for a period of eight months when stored in a refrigerator at 10° C.

The spore suspension was diluted so as to contain 20,000,000 per ml. One ml. was added to 100 ml. of chlorine disinfectant, thus giving a count of 1,000,000 per 5 ml. of test solution. It was found that in these very heavy spore suspensions excessive agitation caused the spores to clump, thereby resulting in reductions in the initial spore plate count. These reductions in count were, to a certain extent, averted by avoiding excessive stirring. This was accomplished by gently drawing the suspension up in a 1 ml. pipette a few times instead of mixing the suspension by shaking the entire container. Even with this precaution, however, clumping occasionally caused reductions in the initial count.

* Butterfield (1932)

Among studies in which spores of Bacillus metiens have been employed may be mentioned those of Myers (1929), Levine, Peterson and Buchanan (1927, 1928), Levine, Toulouse and Buchanan, (1928), Feldman (1938), Cole (1938) and Shillinglaw (1939).

5. Technique of disinfection test.

Preliminary experiments carried out using chlorine solutions made alkaline by the addition of small amounts of sodium hydroxide showed that it was not possible to maintain a constant reaction (pH) in the absence of buffers. The reaction of these solutions (initially alkaline) became more acid on storage. In some cases, increases in acidity were observed which were from one half to one or more pH units. The tendency to become more acid was increased by agitation, and was apparently due to absorption of CO_2 from the air. Since the reaction (pH) did not remain constant it was necessary to employ buffer solutions throughout this study.

After a number of preliminary experiments it was found that $\frac{M}{20}$ buffers were very satisfactory. In order that the final solution of chlorine or chlorine-ammonia mixture should contain a buffer concentration of $\frac{M}{20}$, it was found desirable to add 25 ml. of a $\frac{M}{5}$ buffer solution to 75 ml. of test solution containing the desired amounts of ammonia, chlorine and water.

In some cases it was observed that upon the addition of the chlorine solution to the buffered water, the reaction became more acid. The $\frac{M}{20}$ buffer was not sufficiently concentrated to offset all changes in reaction (pH) due to the chlorine. This was easily overcome, however, by determining the amount of standard sodium hydroxide solution required to bring the solution back to the desired reaction. In order to determine this amount, chlorine was added to the buffered water and potentiometric titrations were made. When the buffered test solutions were prepared and adjusted by adding standard sodium hydroxide solution, no appreciable fluctuations in reaction (pH) occurred throughout the duration of the experiments (in some cases for more than 12 hours).

Control experiments, at room temperature (22-25° C.), in which B. metiens spores were exposed for 24 hours to each of the buffers employed (in the absence of chlorine) showed no appreciable change in bacterial count. In one experiment at pH 10, there was no appreciable reduction in bacterial count even after 12 hours exposure at 50° C. Charlton (1933) reported quite similar results using the same test organism.

All tests were carried out using 250 ml. three-necked Woulfe bottles, each containing 100 ml. of the test solution at the beginning of the experiment. A glass stirrer, driven by an electric motor, was fitted through a stopper in the middle neck of the Woulfe bottle. One of the outside necks

was fitted with a standardized thermometer and the other was stoppered with cotton.

A 25 ml. portion of a sterile $\frac{M}{5}$ buffer was placed in a sterile three-necked Woulfe bottle. The required amount of standard alkali was added to counteract any variation in pH due to additions of chlorine and the desired amount of stock solution* containing 100 p.p.m. NH_3 was added.

A sterile glass stirrer and a thermometer were next inserted and the Woulfe bottle containing the solution was placed in a DeKhotinsky water bath at the desired temperature. The electric electric motor attached to glass stirrer was started, 25 ml. of a standard chlorine solution# were added, care being taken to hold the tip of the pipette below the surface of the solution, and the mixture was stirred for 30 seconds. Within a very few minutes the test solution came to the desired temperature.

At the end of a 15 minute period of contact the electric stirrer was again started, one ml. of a Bacillus metiens spore suspension containing enough cells to give a bacterial count of approximately 1,000,000 per 5 ml. was introduced and the stirring was again continued for 30 seconds.

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* In studying the effect of concentration it was convenient to use a solution containing 200 p.p.m. NH_3.

\# For most of the determinations the standard solution contained 100 p.p.m. available chlorine. Twenty-five ml. of this solution made up to 100 ml. gave a final concentration of 25 p.p.m.

At the end of the desired time intervals the test solution was stirred for 30 seconds, 5 ml. portions were removed by means of sterile bulb pipettes and introduced into 250 ml. Erlenmeyer flasks containing 45 ml. of sterile water with slightly more than enough sodium thiosulfate than was required to neutralize the chlorine contained in the 5 ml. portions. Charlton (1953) has pointed out that sodium thiosulfate in considerable excess of that required to neutralize the chlorine apparently had no effect on the spores.

Serial dilutions were made in 9 ml. distilled water blanks, 1 ml. portions were plated in duplicate on (Difco) nutrient agar, and colonies were counted after 24 hours incubation at 30° C.

At the end of each experiment, the residual chlorine concentration and the reaction (pH) were determined. There was no evidence of pH change greater than 0.05 pH units in any of the experiments.

The temperature variation was $\pm$ 0.1° C. or less in tests of short duration (under three hours). The tests requiring long exposures (6 to 12 hours) showed a maximum temperature variation of $\pm$ 0.3° C.

The zero count was ascertained by determining the number of viable cells in the spore suspension. All counts were based on the number of bacteria surviving in 5 ml., and spores were considered killed if they failed to produce colonies.

C. Results

1. Effect of concentration of ammonia on the germicidal efficiency of chlorine at various reactions (pH).

Preliminary experiments using 25 p.p.m. available chlorine in solutions buffered at pH 6, 7 and 8 indicated that this concentration of chlorine killed 99 percent of the exposed spores in a very few minutes, namely 2.2, 2.9 and 6.9 minutes respectively. However, when 6 p.p.m. NH_3 was added to 25 p.p.m. available chlorine at pH 8, a greatly increased killing time (over 60 minutes) was indicated. It will be noted that the proportion of available chlorine to ammonia (25:6) is approximately the theoretical ratio required for the production of monochloro-amine.

In order to determine the effect of ammonia concentration on the germicidal efficiency of chlorine, experiments were carried out at pH 5, 6, 7, 8, 9 and 10. Experiments employing 25 p.p.m. available chlorine in $\frac{M}{20}$ buffer solution at six reactions (pH), using different concentrations of ammonia (0.0, 0.5, 2, 6 and 18 p.p.m.) are described below.

The method for the preparation of each buffer is given in the appendix. Control experiments maintained for several hours longer than was required for the germicidal tests showed that the buffer solutions employed had no appreciable chlorine

demand when subjected to the same conditions of temperature, concentration and light as were used in this study.

In the summary tables for each reaction (pH) (1E to 6E inclusive) are presented results of residual chlorine determinations made after 15 minutes and at the end of the experiments. These residuals after 15 minutes were determined on solutions prepared in the same manner as those to which the B. metiens spores were exposed, while the residual chlorine determinations at the end of the experiments were made on the solutions which contained the test organism. The approximate times at which the residual chlorine determinations (at the end of the experiments) were made, are given in Table 7A.

Since the organisms were introduced 15 minutes after the solutions were compounded, the residual chlorine at that time is the initial concentration to which the organisms were subjected.

a. Observations at pH 5. In Table 1, 1A, 1B, 1C and 1D are given the results obtained in a series of experiments employing 25 p.p.m. of chlorine at pH 5 in the absence of ammonia, and in the presence of 0.5, 2, 6, and 18.0 p.p.m. respectively. The reaction was maintained by using a $\frac{M}{20}$ acetate buffer (see appendix). The average percents of survivors are shown in the respective tables and the corresponding graphs Figures 1 to 1D.

At pH 5 in the absence of ammonia the initial chlorine concentration (Table 1E) was 24.2 p.p.m. and the average of the residuals determined at the end of each experiment also was 24.2 p.p.m. Killing times varied from 1.9 to 2.3 minutes with an average of 2.1 minutes.

On the addition of 0.5 p.p.m. of ammonia the initial chlorine concentration was 21.1 p.p.m. and no significant change in residual chlorine was observed during the course of the experiments. There was very little difference in the killing times which fluctuated between 2.5 and 2.8 minutes with an average of 2.6 minutes for the destruction of 99% of the exposed spores.

With 2 p.p.m. of ammonia, as is shown in Table 1B and summarized in Table 1F, the initial chlorine concentration had dropped to 14.0 p.p.m. and the residual at the end of the experiment was 11.4 p.p.m. The killing time of 4.4 minutes was slightly longer than that obtained using 0.5 p.p.m. ammonia.

Increasing the concentration of ammonia to 6 p.p.m. (Table 1C) showed an initial chlorine concentration of 22.6 p.p.m. which had dropped only to an average of 22.0 p.p.m. by the time the experiments were completed. A marked increase in the time to kill 99% of the exposed spores was observed using this concentration of ammonia, the two experiments showing 165 and 170 minutes or an average of 168 minutes.

By still further increasing the ammonia concentration to 18 p.p.m., an initial chlorine concentration of 23.3 p.p.m. was observed with no appreciable change during the course of the experiments. A distinct decrease in killing time over that obtained when employing 6 p.p.m. of ammonia (the theoretical ratio of chlorine to ammonia required for the production of monochloroamine) was observed, namely 97 to 102 minutes with an average of 99 minutes.

It appears therefore that as the ammonia content was increased the time for killing 99 percent of the exposed bacteria also increased until a concentration of 6 p.p.m. of ammonia was reached, beyond this there was a reversal to a shorter killing time.

Examination of Figures 1 to 1D inclusive discloses a rather distinct difference in the curves for the low as compared to the high ammonia concentrations. In Figures 1A and 1B in which the ratio of chlorine to ammonia was high, (12.5 to 1 or more) there is a marked lag followed by a progressive increase in the death rate of the bacteria. In Figures 1C and 1D, where the ratio of chlorine to ammonia is low (4.2 to 1 or less), the lag is relatively insignificant and the survivor curve in each case approaches a straight line.

TABLE 1

RESISTANCE OF B. METIENS SPORES TO CHLORINE AT pH 5.0
(25 p.p.m. Av. Cl.; 20° C.)

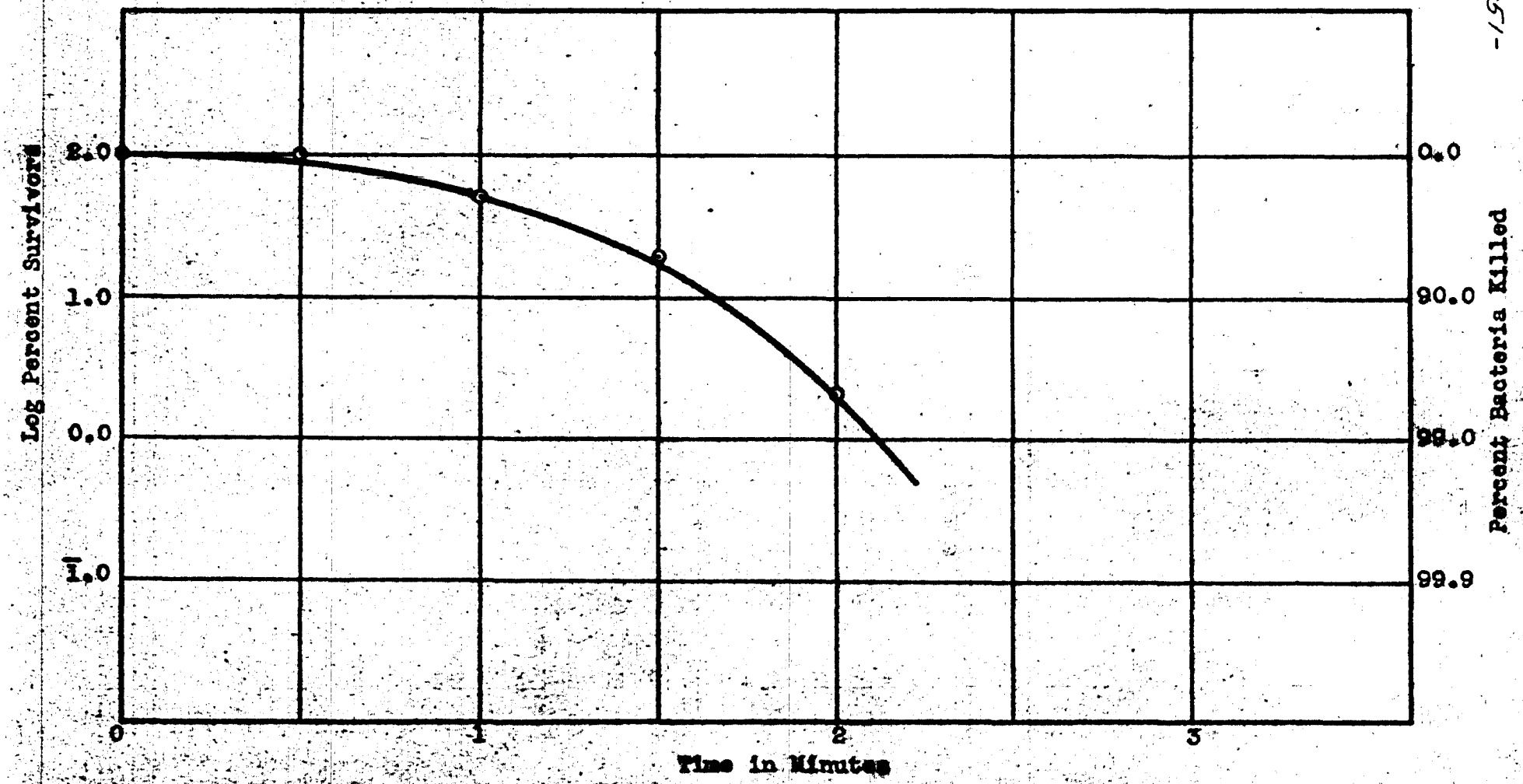
Expt. No. / Date	141 / 2/3/40	146 / 2/10/40	151 / 2/17/40	Average % Survivors	Log Av. % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0.0	750	800	650	100	2.00
0.5	750	750	650	98	1.99
1.0	600	250	225	49	1.69
1.5	285	105	27	18	1.26
2.0	41	5 ##	3	2.2	0.34

				Average
pH **	4.98	5.02	5.00	5.00
Res. Cl. p.p.m. **	24.7	24.1	23.9	24.2
Killing Time (min.) #	2.3	2.0	1.9	2.1

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%
Estimated from survivor curve

Fig. 1

RESISTANCE OF B. METIENS SPORES TO CHLORINE AT pH 5.0
(25 p.p.m. Av. Cl.; 20° C.)



-15-

TABLE 1A

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 5.0 (25 p.p.m. Av. Cl.; 0.5 p.p.m. NH_3; 20° C.)

Expt. No. / Date	140 / 2/3/40	145 / 2/10/40	150 / 2/17/40	Average % Survivors	Log Av. % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0.0	750	800	650	100	2.00
0.5	750	700	700	98	1.99
1.0	330	1,000	500	82	1.91
1.5	155	400	160	32	1.51
2.0	140	175	165	22	1.34
2.5	3.75 ##	16.5	40	2.9	0.46

				Average
pH **	4.98	5.02	5.00	5.00
Res. Cl. p.p.m. **	22.7	21.4	21.3	21.8
Killing Time (min.) #	2.5	2.6	2.8	2.6

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%
Estimated from survivor curve

Fig. 1A

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 5.0
(25 p.p.m. Av. Cl.; 0.5 p.p.m. NH_3; 20° C.)

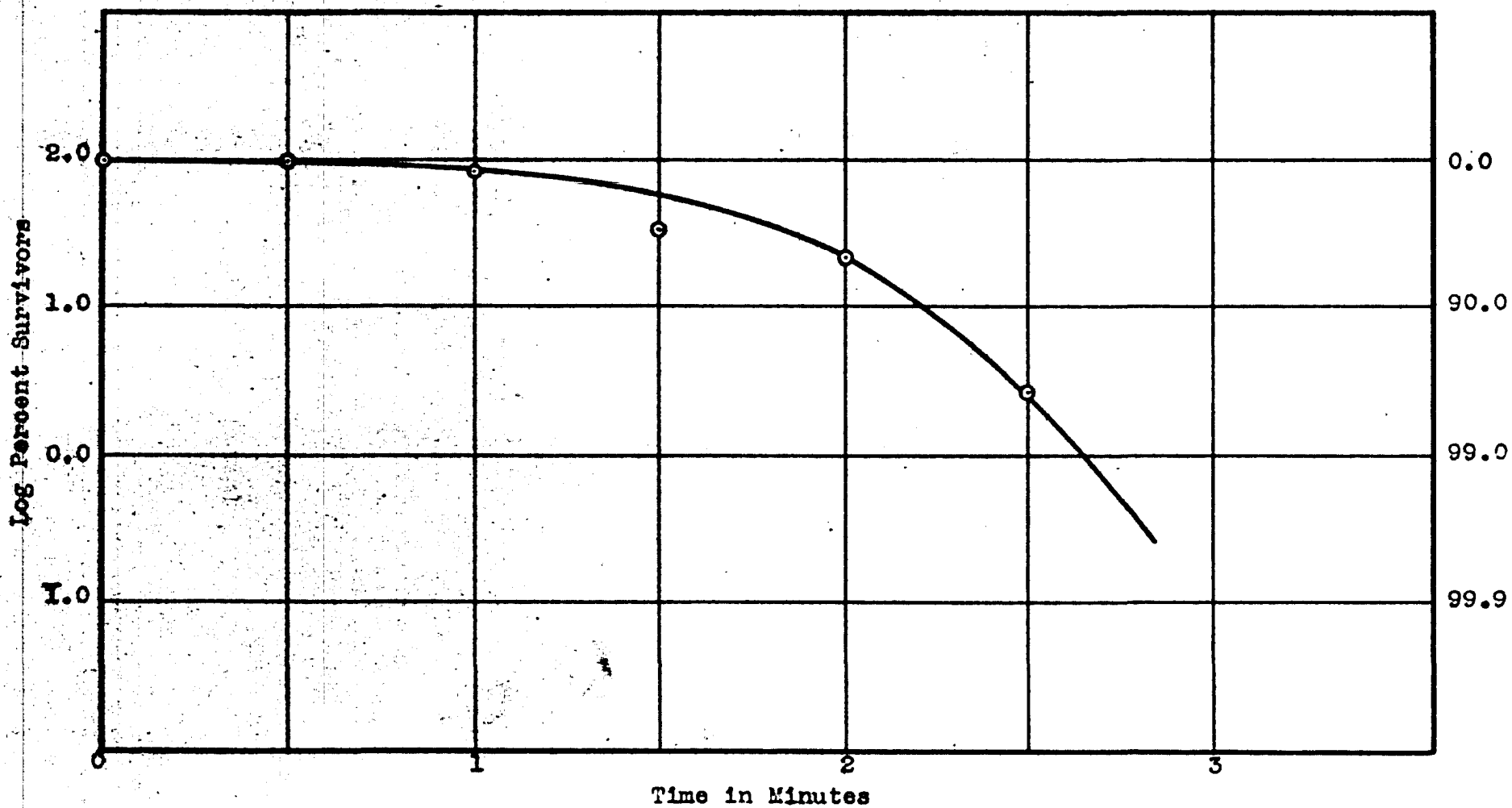


TABLE 1B

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 5.0
(25 p.p.m. Av. Cl.; 2 p.p.m. NH_3; 20° C.)

Expt. No. / Date	144 / 2/10/40	149 / 2/17/40	152 / 2/17/40	Average % Survivors	Log Av. % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0.0	800	500	650	100	2.00
0.5	750	450	700	97	1.99
1.0	850	280	700	90	1.96
1.5	600	320	235	58	1.76
2.0	700	255	290	61	1.79
2.5	320	515	220	59	1.77
3.0	315	150	200	33	1.52
3.5	65	20	130	11	1.04
4.0	38	9	25	3.5	0.54
4.5		7			

				Average
pH **	5.02	5.00	5.00	5.01
Res. Cl. p.p.m. **	11.8	11.3	11.0	11.4
Killing Time (min.) #	4.4	4.3	4.4	4.4

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%

Fig. 1B

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 5.0
(25 p.p.m. Av. Cl.; 2 p.p.m. NH_3; 20° C.)

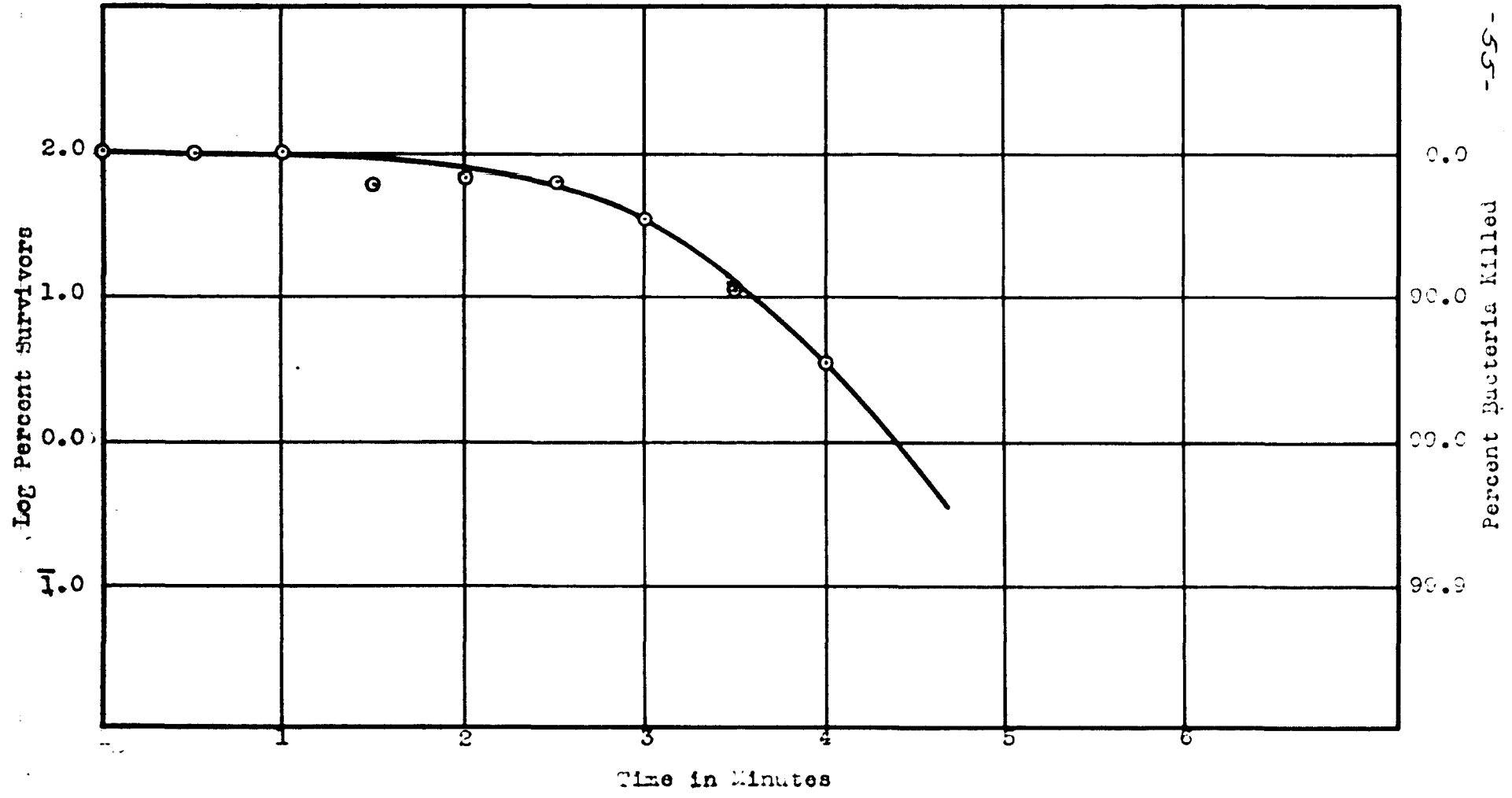


TABLE 1C

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 5.0
(25 p.p.m. Av. Cl.; 6 p.p.m. NH_3; 20° C.)

Expt. No.	148	154	Average	Log Av.
Date	2/10/40	2/17/40	%	%
Exposure Time (in min.)	Surviving Bacteria in Thousands *		Survivors	Survivors
0	800	1,000	100	2.00
20.	850	750	90	1.95
40	400	490	43	1.63
60	230	395	35	1.54
80	250	185	25	1.40
100	36	85	6.5	0.81
120	18	50	3.7	0.57
140	14.5	27.5	2.3	0.36
160	1.2	23.5	1.3	0.11
180	6.5	5.5	0.68	$\bar{1}$.83
200	4.8	4	0.50	$\bar{1}$.70

			Average
pH **	5.02	5.00	5.01
Res. Cl. p.p.m. **	22.0	22.0	22.0
Killing Time (min.) #	165	170	168

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%

Fig. 1C

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 5.0
(25 p.p.m. Av. Cl. & 6 p.p.m. NH_3; 20° C.)

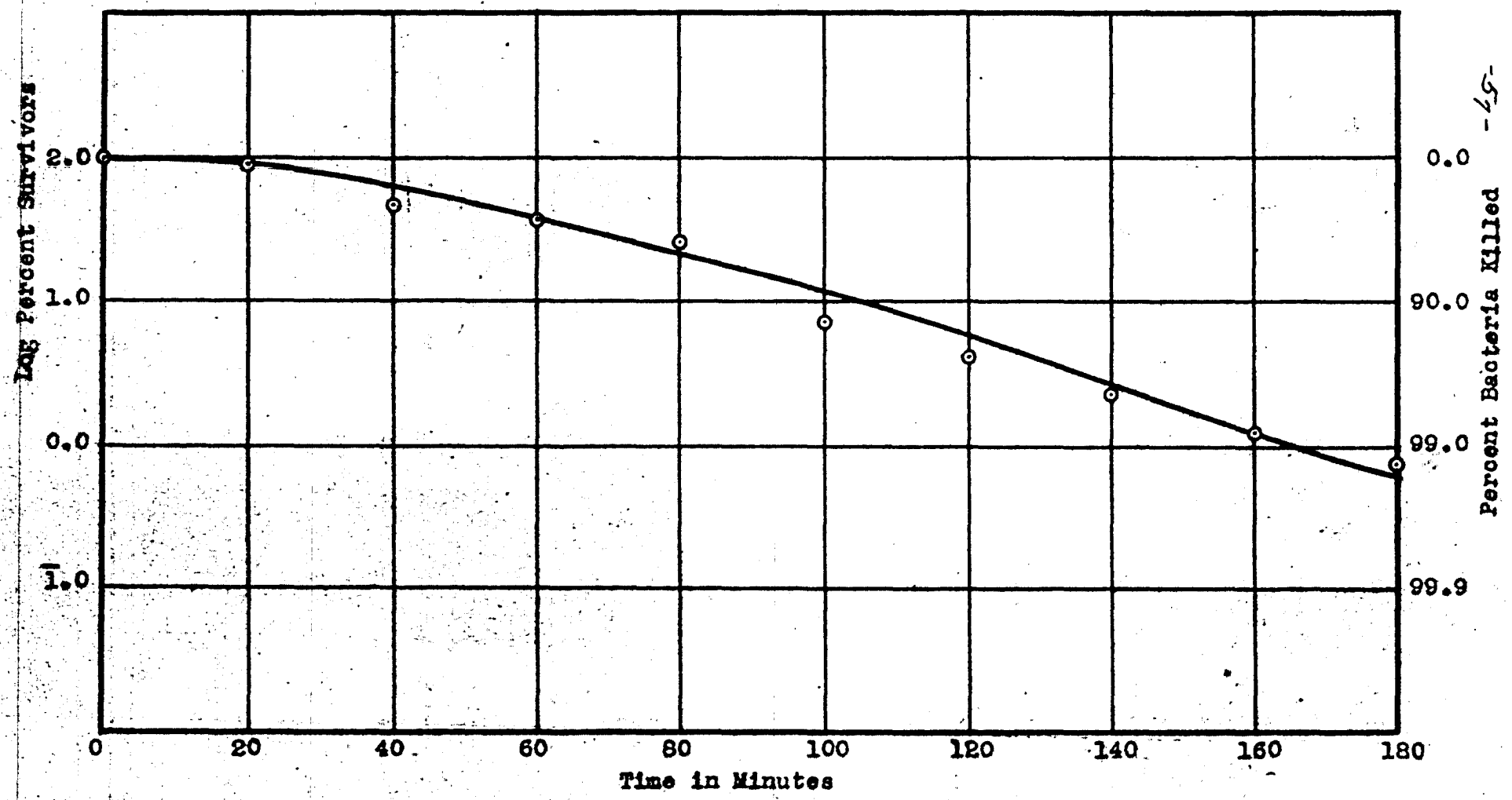


TABLE 1D

RESISTANCE OF *B. METIENS* SPORES TO CHLORINE AND AMMONIA AT pH 5.0
(25 p.p.m. Av. Cl.; 18 p.p.m. HN_3; 20° C.)

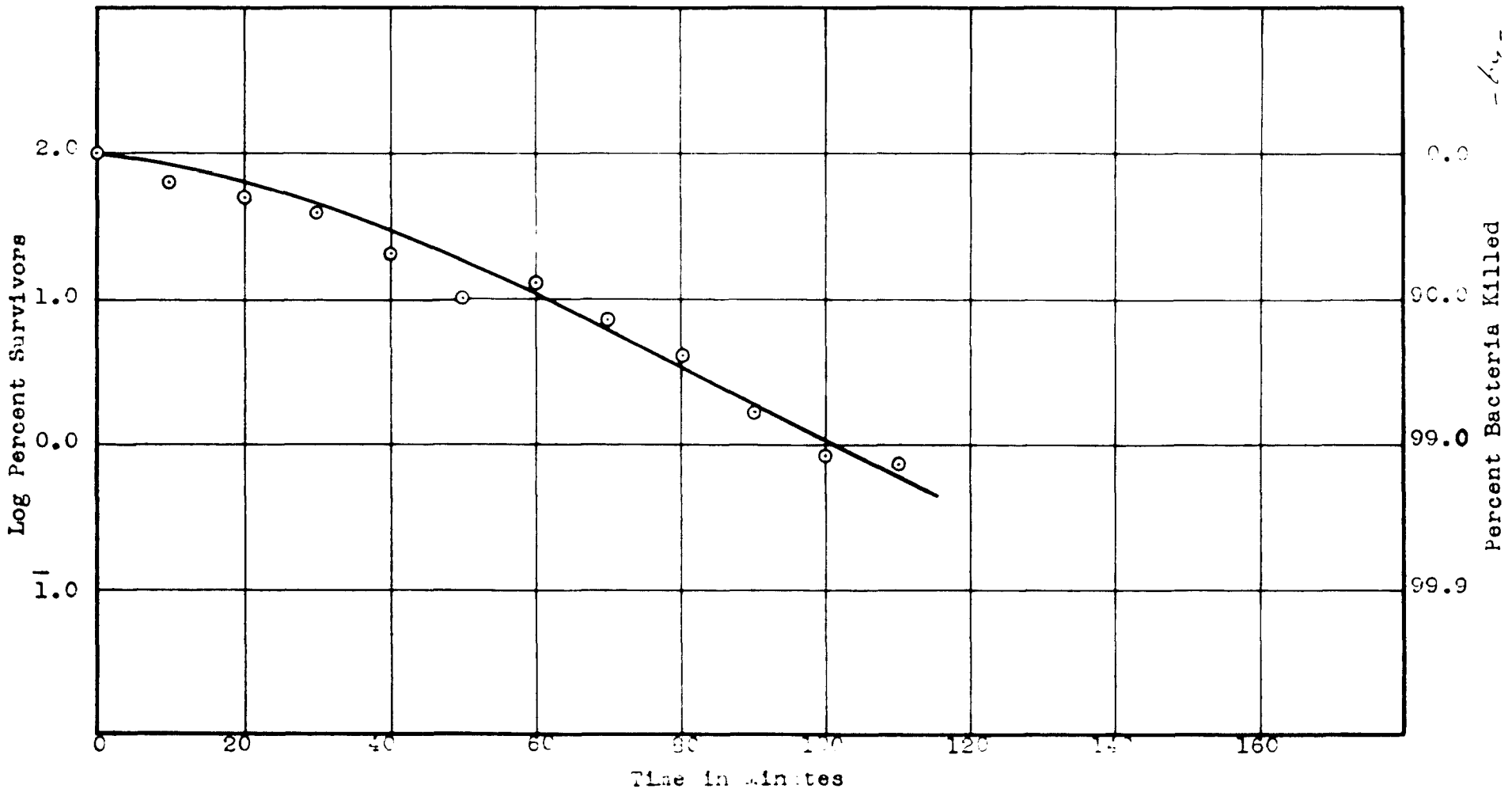
Expt. No. / Date	142 / 2/3/40	147 / 2/10/40	153 / 2/17/40	Average % Survivors	Log Av. % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0	650	800	1,000	100	2.00
10	445	490	550	61	1.79
20	475	285	355	48	1.68
30	250	215	490	38	1.58
40	175	70	185	18	1.26
50	90	18	135	10	1.00
60	115	75	115	13	1.11
70	55	65	55	7.3	0.86
80	40	28.5	24	4.1	0.61
90	14	7	17	1.6	0.20
100	7 ##	3.75	8	0.79	$\bar{1}$.90
110	4 ##	2.75	5.5	0.71	$\bar{1}$.85

				Average
pH **	4.97	5.02	5.00	5.00
Res. Cl. p.p.m. **	24.7	22.5	23.1	23.4
Killing Time (min.) #	102	97	99	99

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%
Estimated from survivor curve

Fig. 1D

RESISTANCE OF *B. METIUM* [illegible] CHLORINE AND AMMONIA AT pH 5.0
(25 p.p.m. Av. Cl.; 18 p.p.m. NH_3; 20° C.)



Results of the experiments detailed in Tables 1 to 1D are summarized in Table 1E. Attention is called to the fact that the reaction remained constant for all these experiments. The killing time, as has previously been pointed out, increased from 2.1 minutes to 168 minutes as the ammonia added rose from 0.0 to 6 p.p.m. but on increasing the ammonia to 18 p.p.m. the killing time fell to 99 minutes.

The residual chlorine data (Table 1E) are of particular interest and attention is called to the fact that except for the solution with 2 p.p.m. of ammonia, which showed a distinct decrease at the end of the experiment, the residual chlorine concentrations were constant. It will be noted that residual chlorine is not a good index of probable germicidal efficiency.

TABLE 1E

SUMMARY OF RESULTS (TABLES 1 TO 1D) AT pH 5.0
(25 p.p.m. Av. Cl. added; 20° C.)

NH_3 (p.p.m.) added	0.0	0.5	2	6	18
Killing* time (min.)	2.1	2.6	4.4	168	99
Residual# Av. Cl. (p.p.m.) after 15 min. contact (initial)	24.2	21.1	14.0	22.6	23.3
Residual#* Av. Cl. (p.p.m.) at end of experiment	24.2	21.8	11.4	22.0	23.4
pH at end of experiment	5.00	5.00	5.01	5.01	5.00
Ratio $\frac{\text{Av. Cl.}}{NH_3}$ added		$\frac{50}{1}$	$\frac{12.5}{1}$	$\frac{4.2}{1}$	$\frac{1.4}{1}$

* Average time required to kill 99 percent exposed spores
\# From Table 7A
#* For approximate times of contact see Table 7A

b. Observations at pH 6. In Tables 2 to 2D are detailed the results of disinfection tests employing 25 p.p.m. chlorine with increasing quantities of ammonia (from 0.0 to 18 p.p.m.). A $\frac{M}{20}$ phosphate buffer was employed to maintain a constant reaction (see appendix). The average percent of survivors for various experiments detailed in Tables 2 to 2D are shown graphically in Figures 2 to 2D respectively.

When employing 25 p.p.m. chlorine without any added ammonia the initial chlorine was 23.4 p.p.m. and the residual at the end of the experiments was 22.3 p.p.m. (Table 2E). The killing time was not significantly different from that observed at pH 5. It may be seen in Table 2 that the killing time varied between 2.2 and 2.5 minutes with an average of 2.3 minutes for the destruction of 99% of the exposed spores as compared with 2.1 minutes obtained at pH 5.

On addition of 0.5 p.p.m. of ammonia the initial chlorine concentration was 19.5 p.p.m. (Table 2E) with no appreciable change during the course of the experiments. Killing times (Table 2A) of 2.4 and 2.6 minutes or an average of 2.5 were obtained, as compared with 2.6 minutes at pH 5, a difference of 0.1 minute being well within the limits of experimental error.

In the presence of .2 p.p.m. of added ammonia the initial chlorine had dropped (Table 2E) to 10.4 p.p.m. but remained constant for the duration of the experiments. Killing times (Table 2B) varied from 4.7 to 4.9 or an average of 4.8 minutes

which was again very close to that obtained at pH 5 namely 4.4 minutes.

When employing 6 p.p.m. ammonia the initial chlorine was 21.8 p.p.m. (Table 2E) and dropped to 20.2 p.p.m. when determined after the completion of the experiments. The killing times obtained in each of the experiments was 85 minutes at pH 6, a distinctly shorter time than the 168 minutes required at pH 5, a decrease in killing time of approximately 50%.

When 18 p.p.m. ammonia had been added to 25 p.p.m. available chlorine the initial chlorine concentration was 23.1 p.p.m. and the residual (at the end(was 22.4 p.p.m. as may be seen in Table 2E. The times to kill 99 percent of the exposed spores varied from 57 to 63 minutes with an average of 59 minutes at pH 6 as compared with 99 minutes previously reported for pH 5.

Perusal of Figure 2, 2A, 2B, 2C and 2D discloses that whereas in Figure 2 to 2B where the ratio of chlorine to ammonia is $\frac{12.5}{1}$ or greater there is evidence of a long lag followed by a curve of increasing slope, that is, increasing death rate, in Figure 2C and 2D the lag period is relatively short and the survival curves each approach a straight line. Similar observations were recorded for the survivor curves at pH 5.

TABLE 2

RESISTANCE OF B. METIENS SPORES TO CHLORINE AT pH 6.0
(25 p.p.m. Av. Cl.; 20° C.)

Expt. No. / Date	72 / 9/30/39	79 / 10/6/39	84 / 10/13/39	Average % Survivors	Log Av. % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0.0	900	750	650	100	2.00
0.5	1,000	850	700	110	2.04
1.0	650	750	410	78	1.89
1.5	385	270	310	42	1.62
2.0	23	35	100	7.4	0.87
2.5			6.5		
3.0			1.75		

				Average
pH **	5.97	5.96	6.00	5.98
Res. Cl. p.p.m. **	22.6	22.1	22.2	22.3
Killing Time (min.) #	2.2	2.3	2.5	2.3

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%

Fig. 2.

RESISTANCE OF *B. METIENS* SPORES TO CHLORINE AT pH 6.0
(25 p.p.m. Av. Cl.; 20° C.)

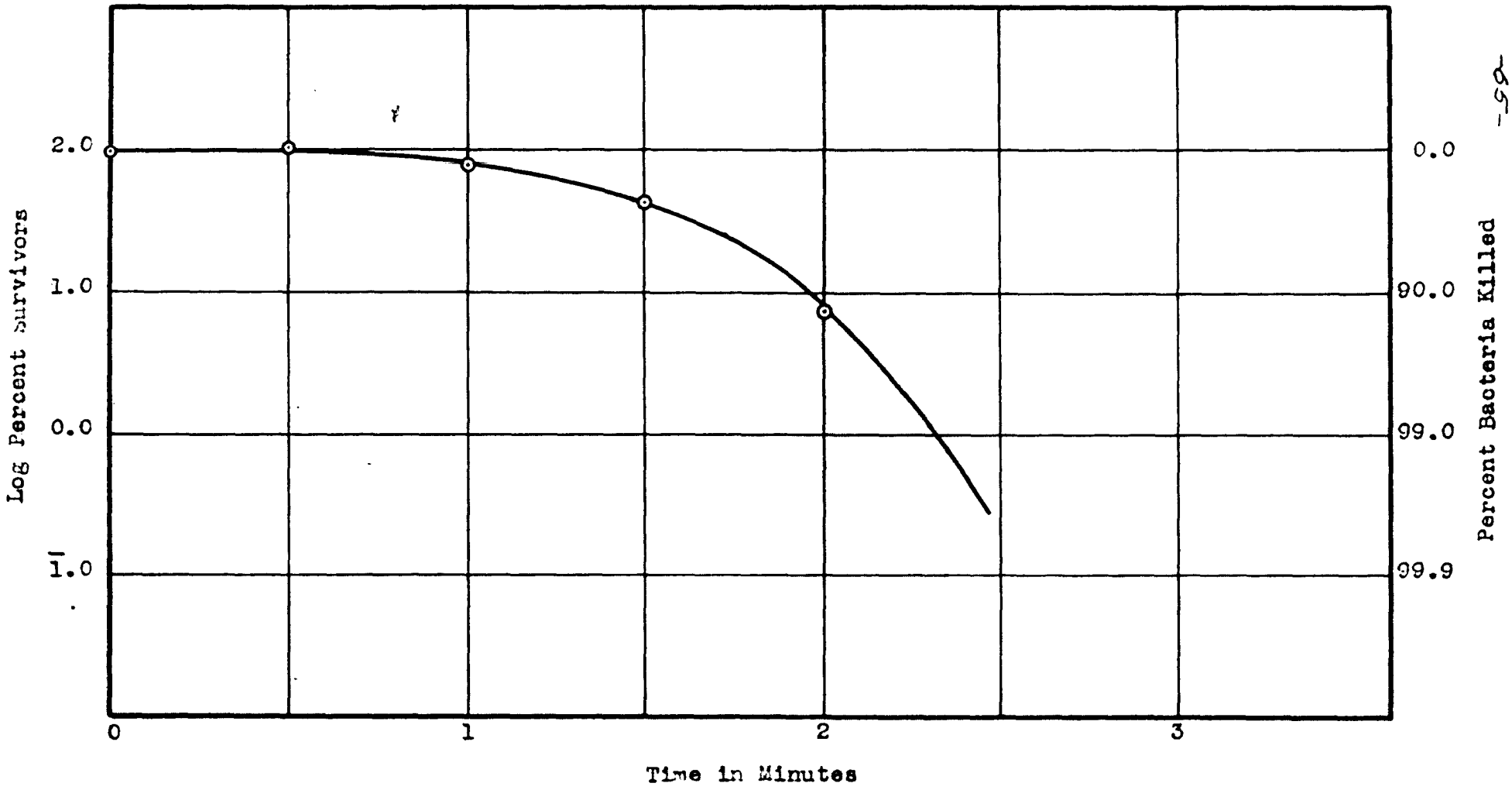


TABLE 2A

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 6.0
(25 p.p.m. Av. Cl.; 0.5 p.p.m. NH_3; 20° C.)

Expt. No.: Date	71 : 9/30/39	78 : 10/6/39	83 : 10/13/39	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0.0	900	750	650	100	2.00
0.5	800	600	475	81	1.91
1.0	650	420	390	63	1.80
1.5	245	280	385	41	1.61
2.0	70	50	80	8.8	0.94
2.5	9 ##	2.5	11	1.0	0.00
3.0			2		

				Average
pH **	5.97	5.97	6.02	5.99
Res. Cl. ** p.p.m.	19.9	19.6	19.6	19.7
Killing Time (min.) #	2.5	2.4	2.6	2.5

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%
Estimated from survivor curve

Fig. 2A

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 6.0
(25 p.p.m. Av. Cl.; 0.5 p.p.m. NH_3; 20° C.)

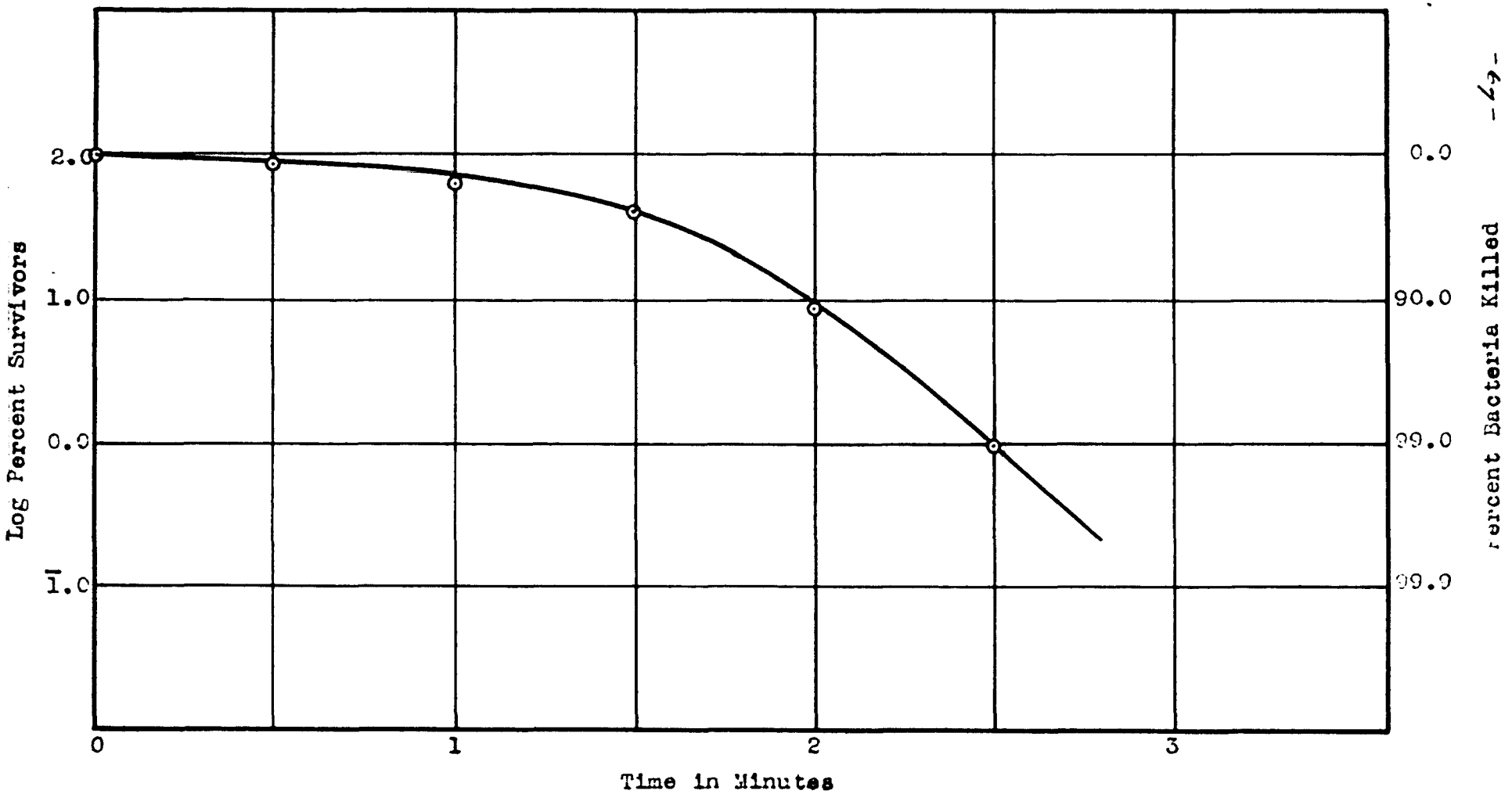


TABLE 2B

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 6.0
(25 p.p.m. Av. Cl.; 2 p.p.m. NH_3; 20° C.)

Expt. No. / Date	70 / 9/30/39	77 / 10/6/39	82 / 10/13/39	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0.0	900	750	650	100	2.00
0.5	900	750	700	103	2.01
1.0	750	500	700	86	1.93
1.5	550	900	550	89	1.95
2.0	650	500	330	63	1.80
3.0	345	320	195	37	1.57
4.0	100	70	33.5	8.5	0.93
5.0	1.25	4	1.75	0.31	$\bar{1}$.49

				Average
pH **	5.97	5.96	6.00	5.98
Res. Cl. p.p.m. **	10.5	9.9	10.3	10.2
Killing Time (min.) #	4.7	4.9	4.7	4.8

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%

Fig. 2B

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 6.0
(25 p.p.m. Av. Cl.; 2 p.p.m. NH_3; 20° C.)

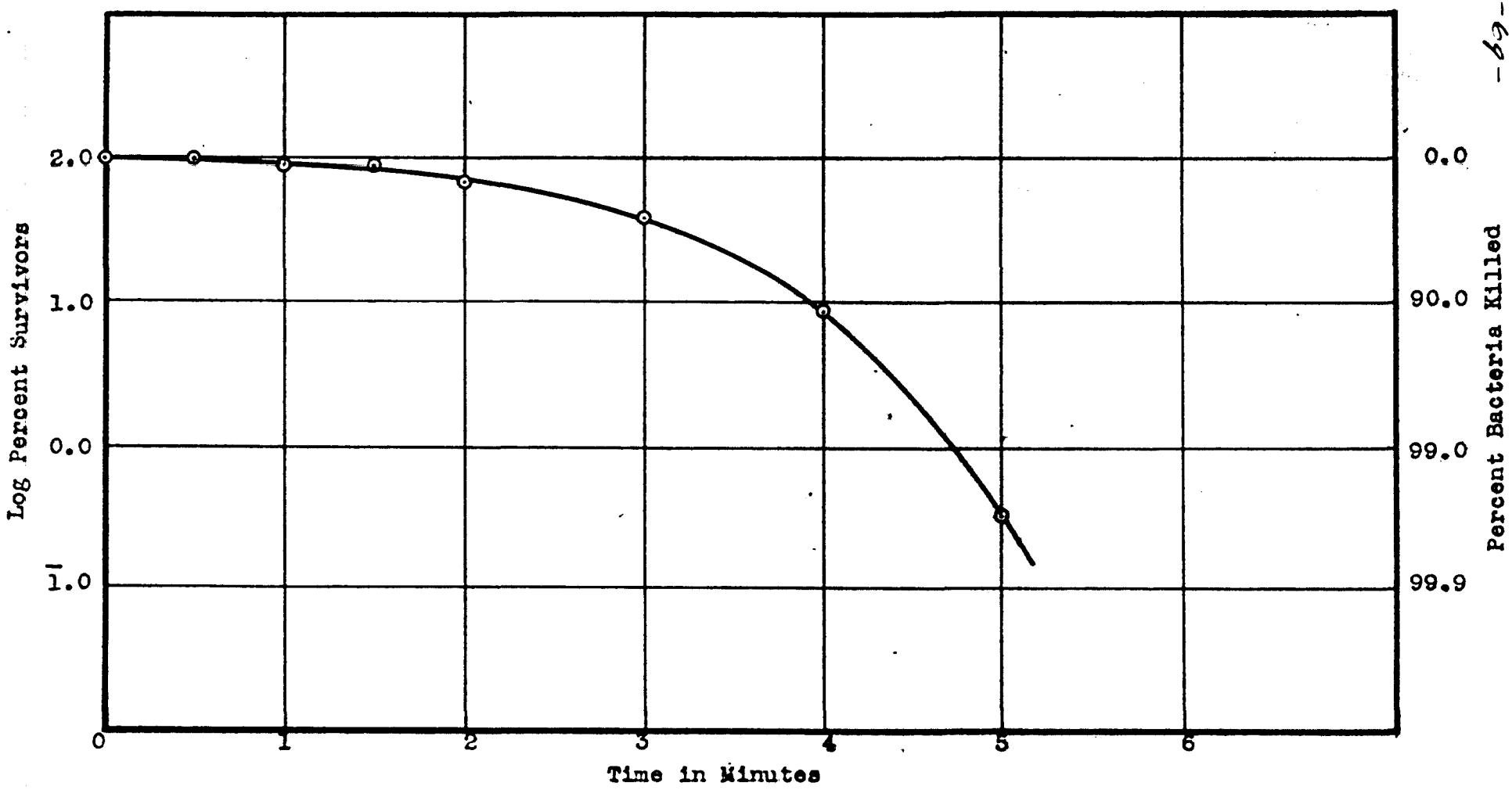


TABLE 2C

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 6.0
(25 p.p.m. Av. Cl.; 6 p.p.m. NH_3; 20° C.)

Expt. No. / Date	68 / 9/30/39	75 / 10/6/39	80 / 10/12/39	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0	900	750	650	100	2.00
10	900	650	550	90	1.95
20	600	455	405	64	1.81
30	275	245	185	30	1.48
40	255	155	160	25	1.40
50	115	95	80	13	1.11
60	60	24	19.5	4.2	0.62
70	11	10	15	1.6	0.20
80	13	9.5	8	1.3	0.11
90	7	4.75	6	0.78	$\bar{1}$.89
100	4.5	4.75	3.75	0.57	$\bar{1}$.76
110	2.5	3.75	2.5	0.38	$\bar{1}$.58

				Average
pH **	5.97	5.96	6.00	5.98
Res. Cl. p.p.m. **	20.3	20.6	19.8	20.2
Killing Time (min.) #	85	85	85	85

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%

Fig. 20

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 6.0
(25 p.p.m. Av. Cl.; 6 p.p.m. NH_3; 20° C.)

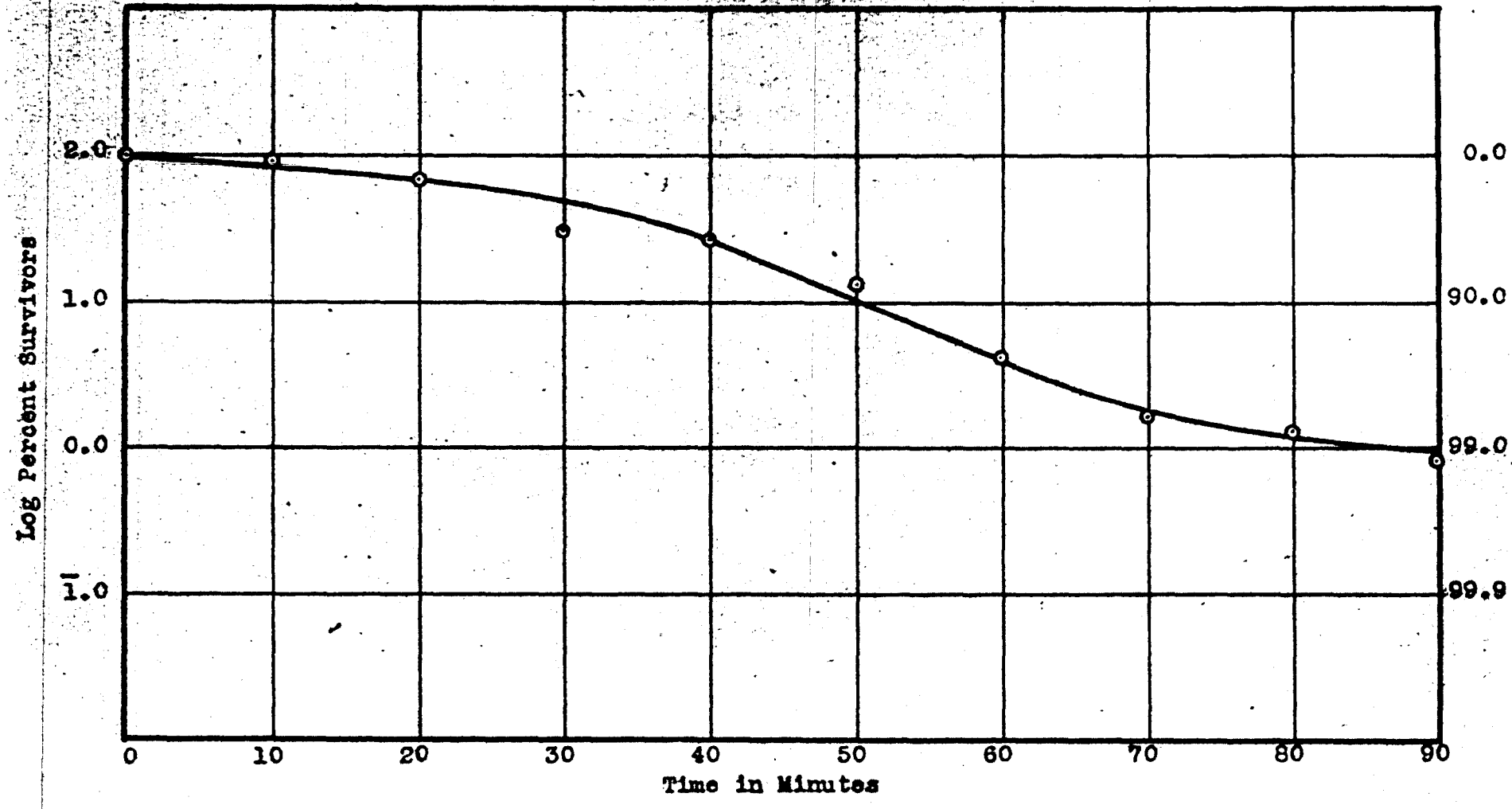


TABLE 2D

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 6.0
(25 p.p.m. Av. Cl.; 18 p.p.m. NH_3; 20° C.)

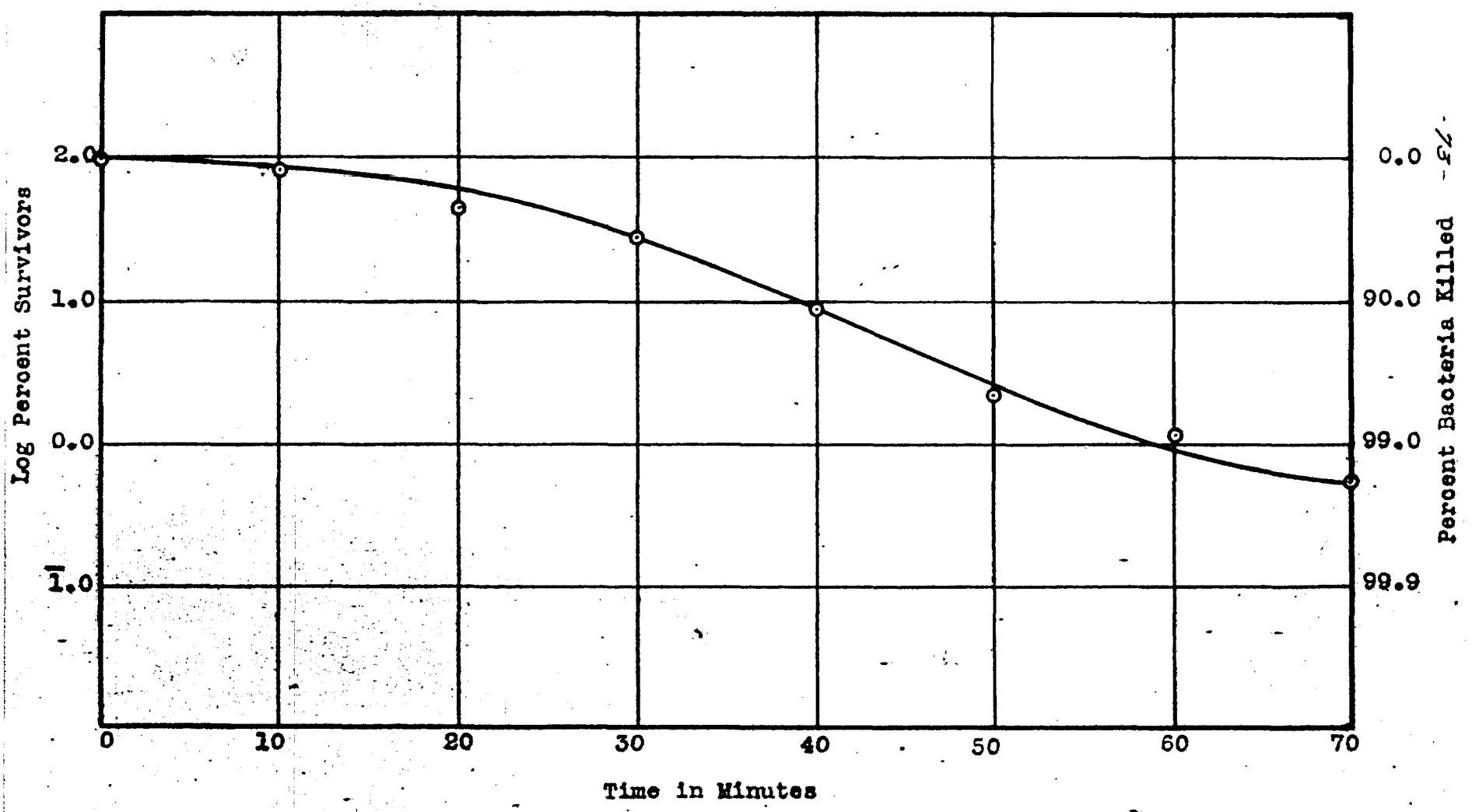
Expt. No. / Date	69 / 9/30/39	76 / 10/6/39	81	Average % Survivors	Log Av. % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0	900	750	650	100	2.00
10	650	465	650	78	1.89
20	325	240	355	45	1.65
30	240	240	140	27	1.43
40	85	37.5	70	8.5	0.93
50	13.5	17.5	16	2.1	0.32
60	10.5	5	0	1.1	0.04
70	3	4.5	4.25	0.53	$\bar{1}$.72

				Average
pH **	6.02	5.96	6.00	5.99
Res. Cl. ** p.p.m.	22.8	22.1	22.3	22.4
Killing Time (min.) #	57	57	63	59

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%

Fig. 2D

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 6.0
(25 p.p.m. Av. Cl.; 18 p.p.m. NH_3; 20° C.)



In Table 2E are summarized the results of experiments presented in Tables 2 to 2D. It will be noted that the pH was constant varying from 5.98 to 5.99. Attention is called to the fact that after the first 15 minute period of contact, the residual available chlorine remained fairly constant. It may be seen that with small concentrations of ammonia up to 2 p.p.m., the killing time increased slowly from 2.3 minutes, in the absence of ammonia, to 2.5 on the addition of 0.5 p.p.m. and to 4.8 minutes with 2 p.p.m. ammonia, while the residual chlorine (after 15 minutes, the time when the spores were added), decreased, the concentrations being 23.4, 19.5 and 10.4 p.p.m. respectively. It might appear then that the killing time increased inversely with the concentration of residual chlorine, but on further addition of ammonia, however, this phenomenon no longer holds true. Thus on the addition of 6 p.p.m. of ammonia the initial chlorine (after 15 minutes contact) increased to 21.8 p.p.m. whereas the killing time rose to 35 minutes and on further increase in the ammonia to 18 p.p.m. the initial chlorine rose to 23.1 p.p.m., the killing time dropping to 59 minutes. It may be noted that the residual chlorine is not a dependable measure of the probable germicidal efficiency.

TABLE 2E

SUMMARY OF RESULTS (TABLES 2 to 2D) AT pH 6.0
(25 p.p.m. Av. Cl. added; 20° C.)

NH_3 (p.p.m.) added	0.0	0.5	2	6	18
Killing Time* (min.)	2.3	2.5	4.8	85	59
Residual# Av. Cl. (p.p.m.) after 15 min. contact (initial)	23.4	19.5	10.4	21.8	23.1
Residual#* Av. Cl. (p.p.m.) at end of experiment	22.3	19.7	10.2	20.2	22.4
pH at end of experiment	5.98	5.99	5.98	5.98	5.99
Ratio $\frac{\text{Av. Cl.}}{NH_3}$ added		$\frac{50}{1}$	$\frac{12.5}{1}$	$\frac{4.2}{1}$	$\frac{1.4}{1}$

* Average time required to kill 99 percent exposed spores.
\# From Table 7A
#* For approximate times of contact see Table 7A

c. Observations at pH 7. The results on the resistance of spores of B. metiens when subjected to the action of 25 p.p.m. of chlorine in the presence of various concentrations of ammonia at a reaction of pH 7 are detailed in Tables 3 to 3D. Concentrations of ammonia employed were the same as in previous experiments, namely, 0.0, 0.5, 2, 6, and 18 p.p.m. The average percents of survivors from these tables are shown graphically in Figures 3 to 3D respectively. All experiments at pH 7 were carried out using $\frac{M}{20}$ phosphate buffer (see appendix).

When employing 25 p.p.m. available chlorine at pH 7, without the addition of ammonia, the initial chlorine was 23.0 p.p.m. and the average residual determined at the end of each experiment was 22.0 p.p.m. (Table 3E). Killing times varied from 2.9 to 3.2 minutes with an average of 3.0 minutes. This was higher (approximately 50 percent) than that obtained at more acid reactions, pH 6 and pH 5.

The addition of 0.5 p.p.m. of ammonia showed initial chlorine concentration of 10.2 p.p.m. (Table 3E) with no appreciable change during the course of the experiments. A slight increase in the killing time above that obtained in the absence of ammonia was observed, namely to 3.3 minutes.

On addition of 2 p.p.m. of ammonia to 25 p.p.m. of chlorine at pH 7, the initial chlorine was 8.1 p.p.m. with no appreciable change during the course of the experiments.

The killing time was still further increased as shown in Table 3B, the times for the individual experiments varying between 6.3 and 6.7 minutes or an average of 6.5 minutes.

Employing 6 p.p.m. of ammonia with 25 p.p.m. available chlorine (the theoretical ratio required for the production of monochloro-amine) showed an initial chlorine concentration of 23.6 p.p.m. with a slight drop during the course of the experiments to an average of 21.1 p.p.m. at the end. Killing times (Table 3C) showed somewhat greater variations than were obtained in experiments previously described, ranging from 82 to 96 minutes with an average of 89 minutes, a killing time which is not significantly different from that obtained with the same concentration of ammonia and chlorine at pH 6.

Increasing the ammonia concentration to 18 p.p.m. gave an initial chlorine concentration of 23.4 p.p.m. with no appreciable change during the experiments (Table 3E). Killing times (Table 3D) varied from 80 to 89 minutes with an average of 84 minutes. This killing time was not significantly different from that obtained with 6 p.p.m. ammonia at pH 7 but it was distinctly higher than the killing time obtained at pH 6 (59 minutes) when employing 18 p.p.m. of ammonia and 25 p.p.m. chlorine.

At pH 7 when employing 6 and 18 p.p.m. ammonia there was very little difference in the killing times which were 89 and 84 minutes respectively. This is distinctly different from

what was observed at pH 5 and pH 6 when using these higher concentrations of ammonia, at these reactions (pH) it will be recalled that the larger concentrations of ammonia (18 p.p.m.) showed a distinct drop in the killing time as compared with 6 p.p.m. ammonia when added to 25 p.p.m. available chlorine.

As has been previously noted, the shape of the survivor curves are characteristically different when high (6 and 18 p.p.m.) and low (2 p.p.m. or less) ammonia are employed. Referring to Figures 3 to 3D, it may be seen that when a high ratio of available chlorine to ammonia (12.5 to 1 or greater) is added the survivor curves show a long lag followed by a curve of increasing slope (increasing death rate) whereas with low ratios (4.2 to 1 or less) the lag is materially reduced and the death rate is quite constant, approaching a straight line.

TABLE 3

RESISTANCE OF B. METIENS SPORES TO CHLORINE AT pH
(25 p.p.m. Av. Cl.; 20° C.)

Expt. No. / Date	10 / 7/25/39	46 / 8/24/39	52 / 9/12/39	57 / 9/16/39	62 / 9/22/39	67 / 9/24/39	12/
Exposure Time (in min.)	Surviving Bacteria in Thousands *						
0.0	1,000	405	950	650	1,000	700	
0.5	1,000	220	800	700	700	500	1,
1.0	750	255	600	480	750	700	
2.0	300	130	325	275	365	370	
3.0	4.5	3.5	6.5	5	16.5	10.5	
pH **	7.01	7.00	6.95	6.99	7.01	6.96	
Res. Cl. p.p.m. **	22.3	22.3	21.3	22.9	21.8	22.1	
Killing Time (min.) #	2.9	3.0	3.0	3.0	3.1	3.1	

* Surviving bacteria (in thousands) per 5 ml.
** At the end of experiment
\# Time for killing 99%

TABLE 3

TANCE OF B. METIENS SPORES TO CHLORINE AT pH 7.0
(25 p.p.m. Av. Cl.; 20° C.)

52	57	62	67	122	184	Average % Survivors	Log Average % Survivors
9/12/39	9/16/39	9/22/39	9/24/39	12/8/39	4/22/40		
Surviving Bacteria in Thousands *							
950	650	1,000	700	850	850	100	2.00
800	700	700	500	1,100	950	91	1.96
600	480	750	700	650	700	76	1.88
325	275	365	370	370	350	39	1.59
6.5	5	16.5	10.5	18	7	1.1	0.04

						Average
6.95	6.99	7.01	6.96	7.02	7.01	6.99
21.3	22.9	21.8	22.1	20.7	22.8	22.0
3.0	3.0	3.1	3.1	3.2	3.0	3.0

usands) per 5 ml.

Fig. 3

RESISTANCE OF B. METIENS SPORES TO CHLORINE AT pH 7.0
(25 p.p.m. Av. Cl.; 20° C.)

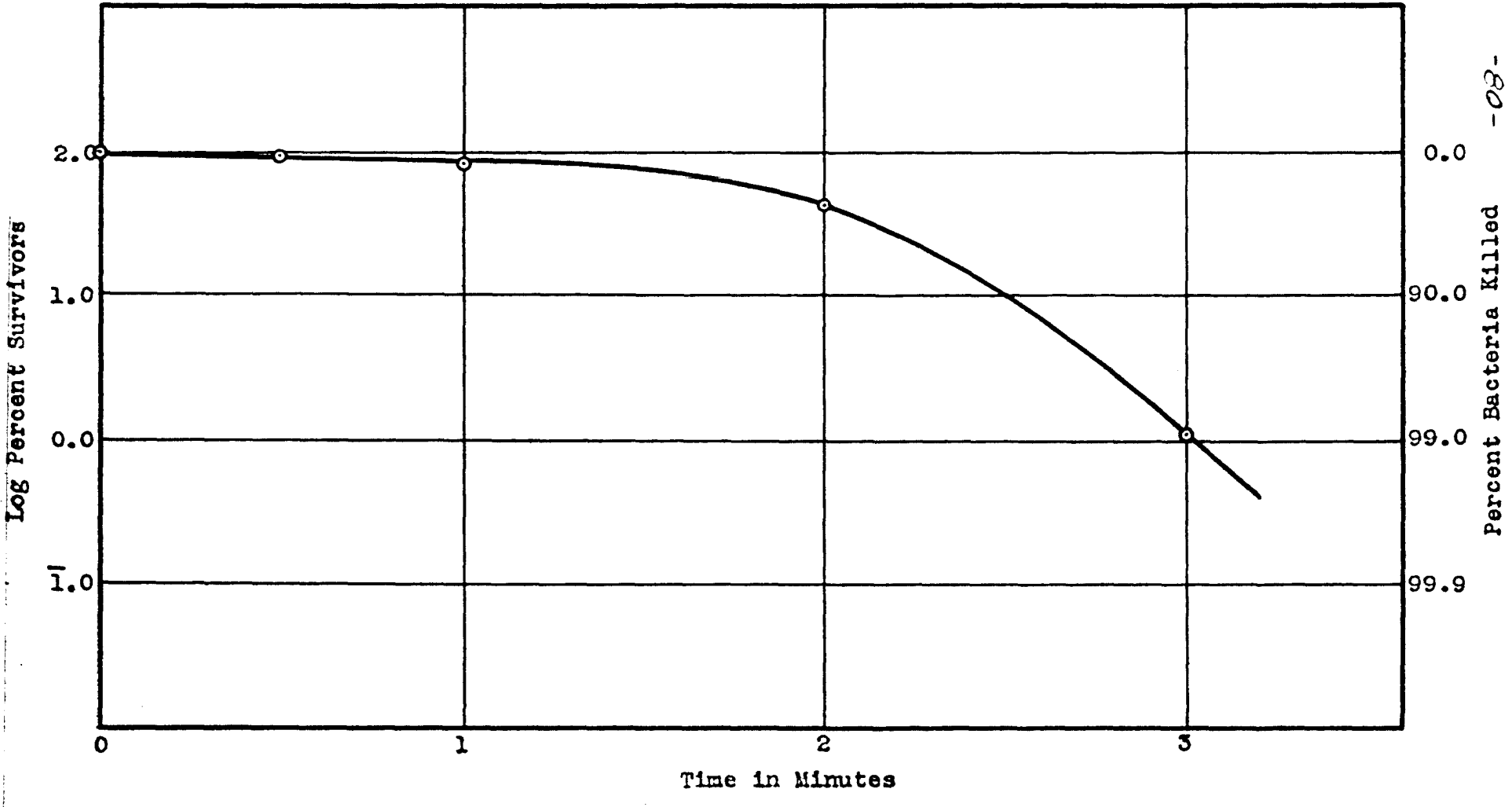


TABLE 3A

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 7.0
(25 p.p.m. Av. Cl.; 0.5 p.p.m. NH_3; 20° C.)

Expt. No. / Date	56 / 9/16/39	61 / 9/22/39	66 / 9/24/39	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0.0	650	1,000	700	100	2.00
0.5	500	800	750	88	1.94
1.0	650	850	485	85	1.93
2.0	175	405	235	34	1.53
3.0	14	21.5	18	2.3	0.36
4.0			2.5		

				Average
pH **	7.00	7.00	6.95	6.98
Res. Cl. p.p.m. **	18.8	19.0	19.2	19.0
Killing Time (min.) #	3.3	3.3	3.3	3.3

* Surviving bacteria (in thousands) per 5 ml.
** At the end of experiment
\# Time for killing 99%

Fig. 3A

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 7.0
(25 p.p.m. Av. Cl.; 0.5 p.p.m. NH_3; 20° C.)

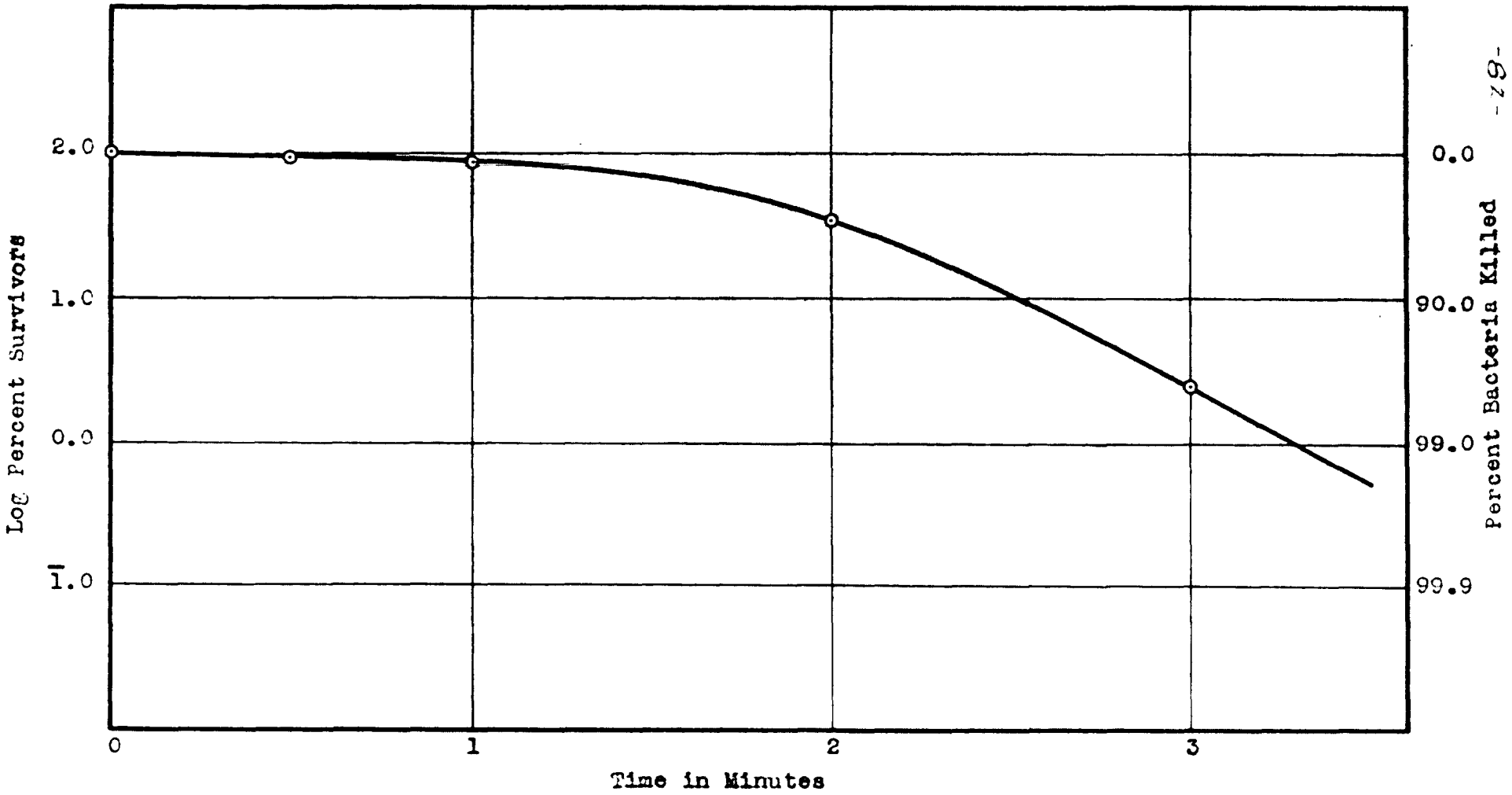


TABLE 3B

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 7.0
(25 p.p.m. Av. Cl.; 2 p.p.m. NH_3; 20° C.)

Expt. No. / Date	55 / 9/16/39	60 / 9/22/39	65 / 9/24/39	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0	650	1,000	700	100	2.00
1	750	1,000	600	100	2.00
2	600	750	800	94	1.97
3	365				
4	215	400	335	40	1.60
5	125				
6	21.5	19.5	20.5	2.7	0.43

				Average
pH **	7.00	7.01	6.98	7.00
Res. Cl. p.p.m. **	8.8	8.6	8.7	8.7
Killing Time (min.)	6.7	6.3	6.6	6.5

* Surviving bacteria (in thousands) per 5 ml.
** At the end of experiment
\# Time for killing 99%

Fig. 3B

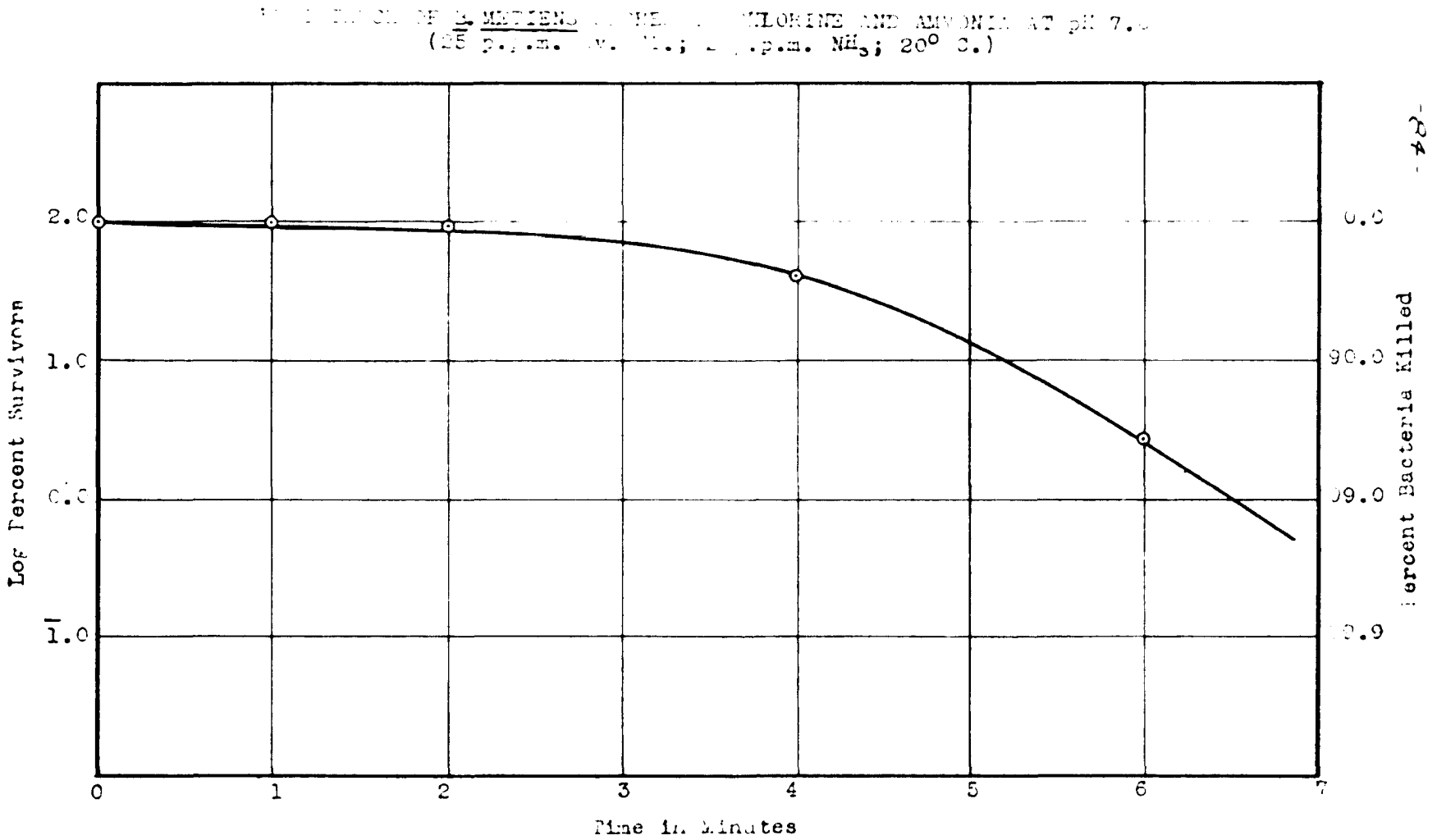

Log Percent Survivors
2.0
1.0
0.0
1.0
0
1
2
3
4
5
6
7
Time in Minutes
90.0
99.0

TABLE 3C

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH. 7.0
(25 p.p.m. Av. Cl.; 6 p.p.m. NH_3; 20° C.)

Expt. No.	48	53	58	63	Average	Log Average
Date	9/12/39	9/16/39	9/22/39	9/24/39	% Survivors	% Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *					
0	600	650	700	700	100	2.00
10	460	500	550	650	82	1.91
20	435	435	485	550	72	1.86
30	385	405	310	265	52	1.72
40	150	165	205	215	28	1.45
50	115	80	80	135	15	1.18
60	47.5	27.5	80	115	9.8	0.99
70	2.5	16	80	65	5.8	0.76
80	6.5	9	14	15.5	1.7	0.23
90	2.5 ##	2.25	7.5	7.5	0.75	$\bar{1}$.88
100	0.95 ##	4	5	5	0.55	$\bar{1}$.74

					Average
pH **	7.00	6.99	6.98	6.98	6.99
Res. Cl. p.p.m. **	21.3	21.7	21.5	20.0	21.1
Killing Time (min.) #	82	83	93	96	89

* Surviving bacteria (in thousands) per 5 ml.
** At the end of experiment
\# Time for killing 99%

Fig. 3C

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH. 7.0
(25 p.p.m. Av. Cl.; 6 p.p.m. NH_3; 20° C.)

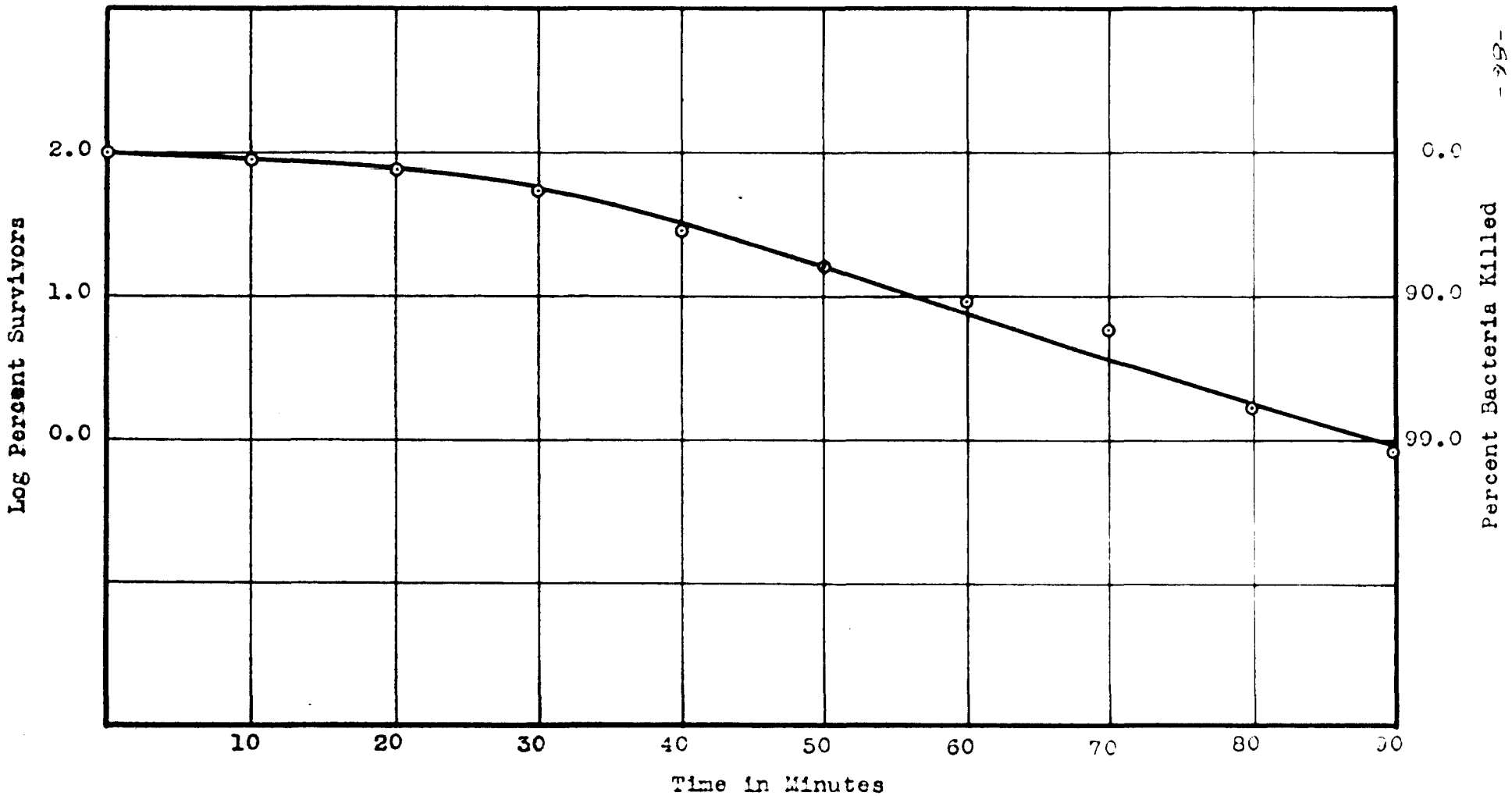


TABLE 3D

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 7.0
(25 p.p.m. Av. Cl.; 18 p.p.m. NH_3; 20° C.)

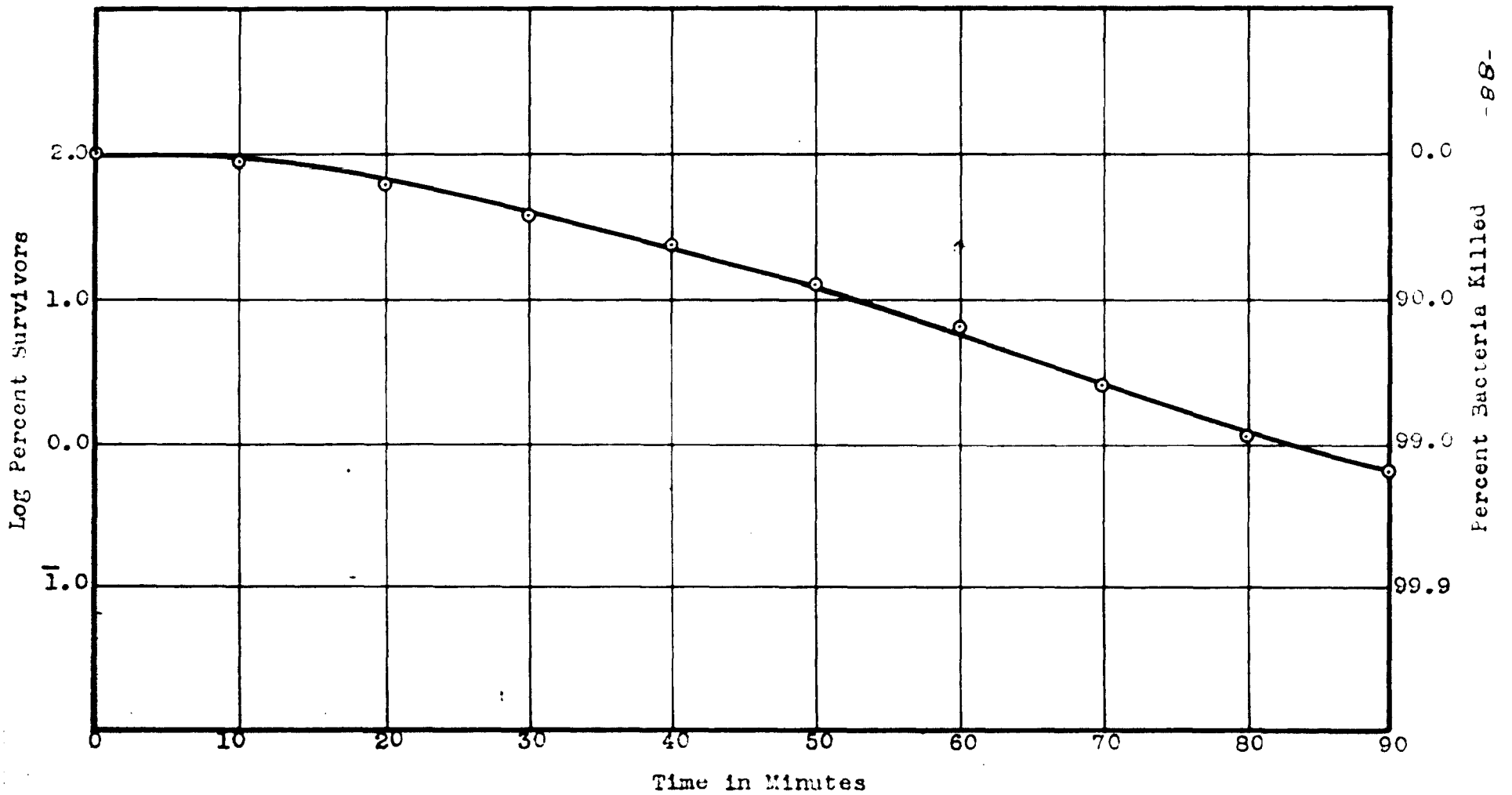
Expt. No. / Date	49 / 9/12/39	54 / 9/16/39	59 / 9/22/39	64 / 9/24/39	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *					
0	950	650	700	700	100	2.00
10	750	600	550	600	84	1.92
20	460	375	475	390	58	1.76
30	270	295	285	210	36	1.56
40	130	165	220	145	23	1.36
50	95	75	60	110	12	1.08
60	46	24	46	75	6.5	0.81
70	11	15	19.5	23	2.4	0.38
80	8.5	5	8.5	11	1.1	0.04
90	5.5	0.75	6.5	6.5	0.64	$\bar{1}.81$

					Average
pH **	6.98	6.99	6.95	6.98	6.98
Res. Cl. p.p.m. **	23.8	23.5	24.5	23.2	23.8
Killing Time (min.) #	80	81	86	89	84

* Surviving bacteria (in thousands) per 5 ml.
** At the end of experiment
\# Time for killing 99%

Fig. 3D

RATE OF DEATH OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 7.0
(25 p.p.m. Av. Cl.; 18 p.p.m. NH_3; 20° C.)



The results detailed in Tables 3 to 3D are summarized in Table 3E. Attention is called to the fact that the reaction (pH) remained very near pH 7 in all of the test solutions for the duration of each experiment.

As the ratio of available chlorine to ammonia (added) decreased from infinity to 12.5/1, the residual chlorine (after 15 minutes contact, the time when the spores were added) also decreased from 23.0 to 8.1 p.p.m. and the killing time increased from 3.0 to 6.5 minutes. By further decreasing the ratio of chlorine to ammonia, that is with higher concentrations of added ammonia, this phenomenon was reversed. The initial available chlorine markedly increased (23.6 p.p.m.) when 6.0 p.p.m. of ammonia was added (chlorine to ammonia equals 4.2 to 1) and 23.0 p.p.m. when 18 p.p.m. of ammonia (chlorine/ammonia equals 1.4 to 1) were added to the 25 p.p.m. of available chlorine, but the killing times now rose to 89 and 84 minutes respectively which were considered not to be significantly different. Here again it may be noted that the residual chlorine is not a dependable index of probable germicidal efficiency.

TABLE 3E

SUMMARY OF RESULTS (TABLES 3 TO 3D) AT pH 7.0
(25 p.p.m. Av. Cl. added; 20° C.)

NH_3 (p.p.m.) added	0.0	0.5	2	6	18
Killing* time (min.)	3.0	3.3	6.5	89	84
Residual# Av. Cl. (p.p.m.) after 15 min. contact (initial)	23.0	19.2	8.1	23.6	23.4
Residual#* Av. Cl. (p.p.m.) at end of experiment	22.0	19.0	8.7	21.1	23.8
pH at end of experiment	6.99	6.98	7.00	6.99	6.98
Ratio $\frac{\text{Av. Cl. added}}{NH_3}$		$\frac{50}{1}$	$\frac{12.5}{1}$	$\frac{4.2}{1}$	$\frac{1.4}{1}$

* Average time required to kill 99 percent exposed spores
\# From Table 7A
#* For approximate times of contact see Table 7A

d. Observations at pH 8. The results of the experiments on the resistance of B. metien spores to chlorine at pH 8 and various concentrations of ammonia are detailed in Tables 4 to 4D. The corresponding survivor curves are shown graphically in Figures 4 to 4D. Ammonia concentrations employed were the same as in previous experiments, namely, 0.0, 0.5, 2, 6 and 18 p.p.m. The buffer consisted of $\frac{M}{20}$ phosphate and the method of preparation is given in the appendix.

When employing 25 p.p.m. chlorine with no added ammonia at pH 8 (Table 4E) the initial chlorine concentration was 22.0 p.p.m. with no appreciable change during the course of the experiments. Killing times (Table 4) varied from 6.9 to 8.3 minutes, with an average of 7.6 minutes. This was distinctly higher than was obtained with the more acid solutions previously described.

The addition of 0.5 p.p.m. of ammonia (Table 4E) reduced the initial chlorine to 18.6 p.p.m. and no significant change was noted for the average of residuals determined at the end of each experiment. Table 4A shows that the killing times ranged from 8.3 to 8.8 minutes with an average of 8.6 minutes.

When 2 p.p.m. ammonia was added the initial chlorine (Table 4E) was 7.5 p.p.m. and the average of chlorine determinations taken at the end of each experiment showed that no appreciable change occured during the experiments.

It may be seen in Table 4B that the killing times for the individual experiments averaged 21 minutes.

With the addition of 6 p.p.m. ammonia to 25 p.p.m. available chlorine at pH 8, it will be noted in Table 4E that an initial chlorine concentration of 22.0 remained rather constant during the experiments. Table 4C shows that the killing times varied from 83 to 89 minutes with an average of 85 minutes.

When the ammonia concentration was increased to 18 p.p.m. the initial chlorine was 22.8 p.p.m. with no significant change during the experiments. Killing times ranged from 100 to 116 minutes with an average of 107 minutes.

It will be noted that at pH 8 the killing time progressively increased with increasing concentrations of ammonia for the entire series, but it is desired to point out particularly that in contrast to what was observed at pH 5 and 6, where the addition of 18 p.p.m. of ammonia resulted in a marked decrease of the killing time as compared with that obtained with 6 p.p.m. of ammonia, and pH 7, where the killing time was not significantly different with these two concentrations of ammonia, at pH 8 the higher concentration of ammonia showed a distinct increase in the killing time.

The shapes of the survivor curves at pH 8 show the same trend as previously described for pH 5, 6 and 7, namely,

that with a ratio of chlorine to ammonia of 12.5/1 or more they show a marked lag followed by an increasing death rate, whereas when the ratios of chlorine to ammonia of approximately 4.2 to 1 and 1.4 to 1 are employed the preliminary lag is very short and the survivor curve simulates a straight line.

TABLE 4

RESISTANCE OF B. METIENS SPORES TO CHLORINE AT pH 8.
(25 p.p.m. Av. Cl.; 20° C.)

Expt. No.	13	17	21	25	31	36	
Date	8/5/39	8/8/39	8/12/39	8/14/39	8/17/39	8/21/39	8/
Exposure Time (in min.)	Surviving Bacteria in Thousands *						
0	950	1,150	1,050	950	1,250	1,250	
2	700	750	900	1,150	900	1,150	
4	275	500	800	650	850	1,000	
6	27.5	42	95	210	115	155	
8	4.25 #	3.5 ##	4	13.5	10.5	7	
pH **	8.01	8.01	7.99	8.00	7.99	8.00	
Res. Cl. P.p.m. **	24.2	23.5	22.3	23.4	22.3	22.4	2
Killing Time (min.)	6.9	7.0	7.4	8.3	7.9	7.7	

* Surviving bacteria (in thousands* per 5 ml.
** At end of experiment
\# Time for killing 99%
Estimated from killing curve

TABLE 4

STANCE OF B. METIENS SPORES TO CHLORINE AT pH 8.0
(25 p.p.m. Av. Cl.; 20° C.)

21	25	31	36	42	Average % Survivors	Log Average % Survivors
8/12/39	8/14/39	8/17/39	8/21/39	8/23/39		
Surviving Bacteria in Thousands *						
1,050	950	1,250	1,250	405	100	2.00
900	1,150	900	1,150	415	87	1.94
800	650	850	1,000	275	72	1.86
95	210	115	155	85	11	1.04
.5 ## 4	13.5	10.5	7	5.5	0.77	$\bar{1}$.89

					Average
1 7.99	8.00	7.99	8.00	7.97	8.00
22.3	23.4	22.3	22.4	22.4	22.9
7.4	8.3	7.9	7.7	8.3	7.6

usands* per 5 ml.

ve

Fig. 4

RESISTANCE OF B. METIENS SPORES TO CHLORINE AT pH 8.0
(25 p.p.m. Av. Cl.; 20° C.)

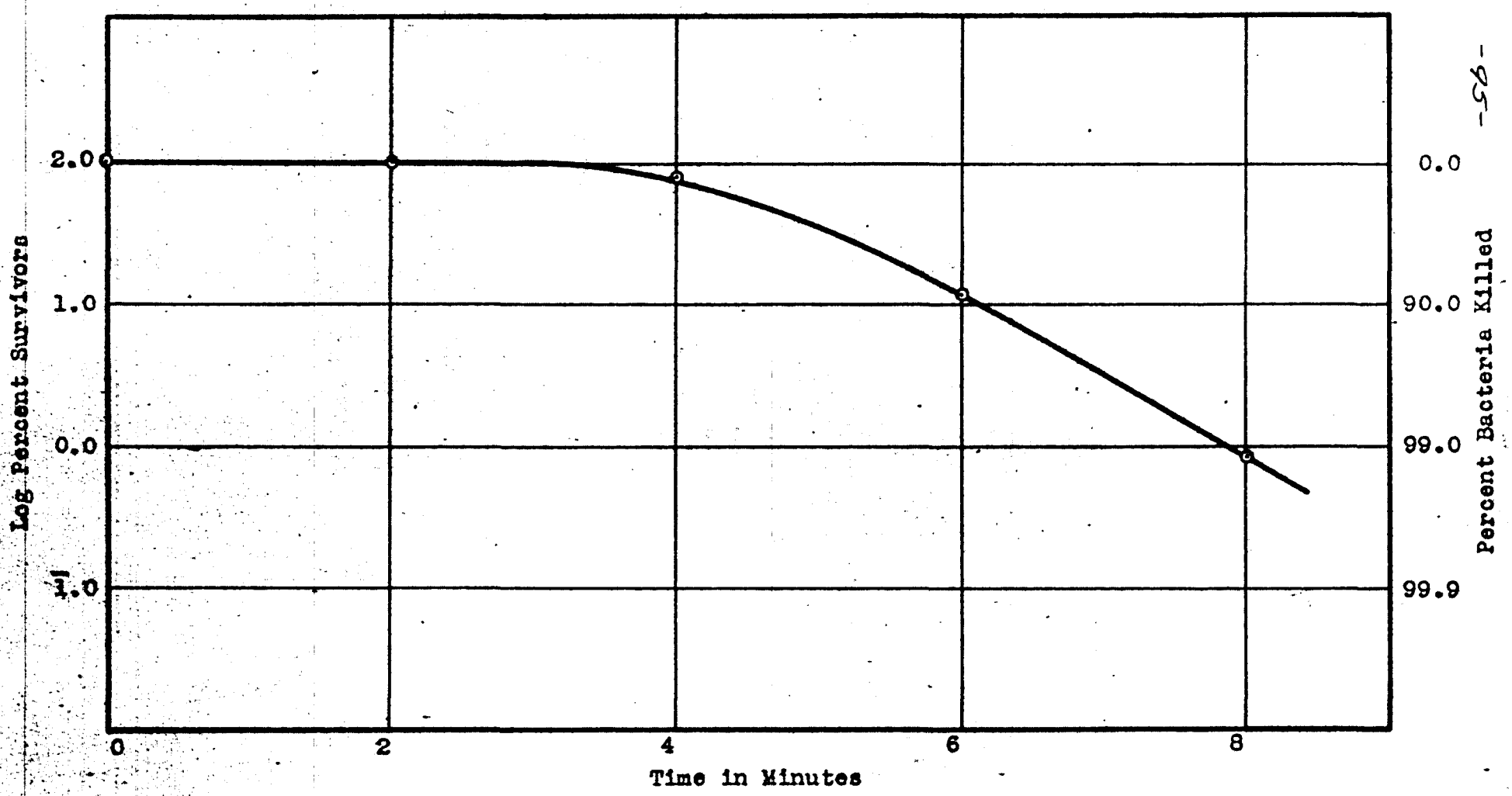


TABLE 4A

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 8.0
(25 p.p.m. Av. Cl.; 0.5 p.p.m. NH_3; 20° C.)

Expt. No. / Date	22 / 8/12/39	32 / 8/17/39	40 / 8/21/39	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0	1,050	1,250	800	100	2.00
2	1,000	1,050	800	93	1.97
4	750	800	650	72	1.86
6	230	280	215	23	1.36
8	15	35	21	2.3	0.36
10		1.75			

				Average
pH **	8.01	7.99	7.99	8.00
Res. Cl. p.p.m. **	18.8	18.8	19.0	18.9
Killing Time (min.) #	8.3	8.8	8.8	8.6

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%

Fig. 4A

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 8.0
(25 p.p.m. Av. Cl.; .5 p.p.m. NH_3; 20° C.)

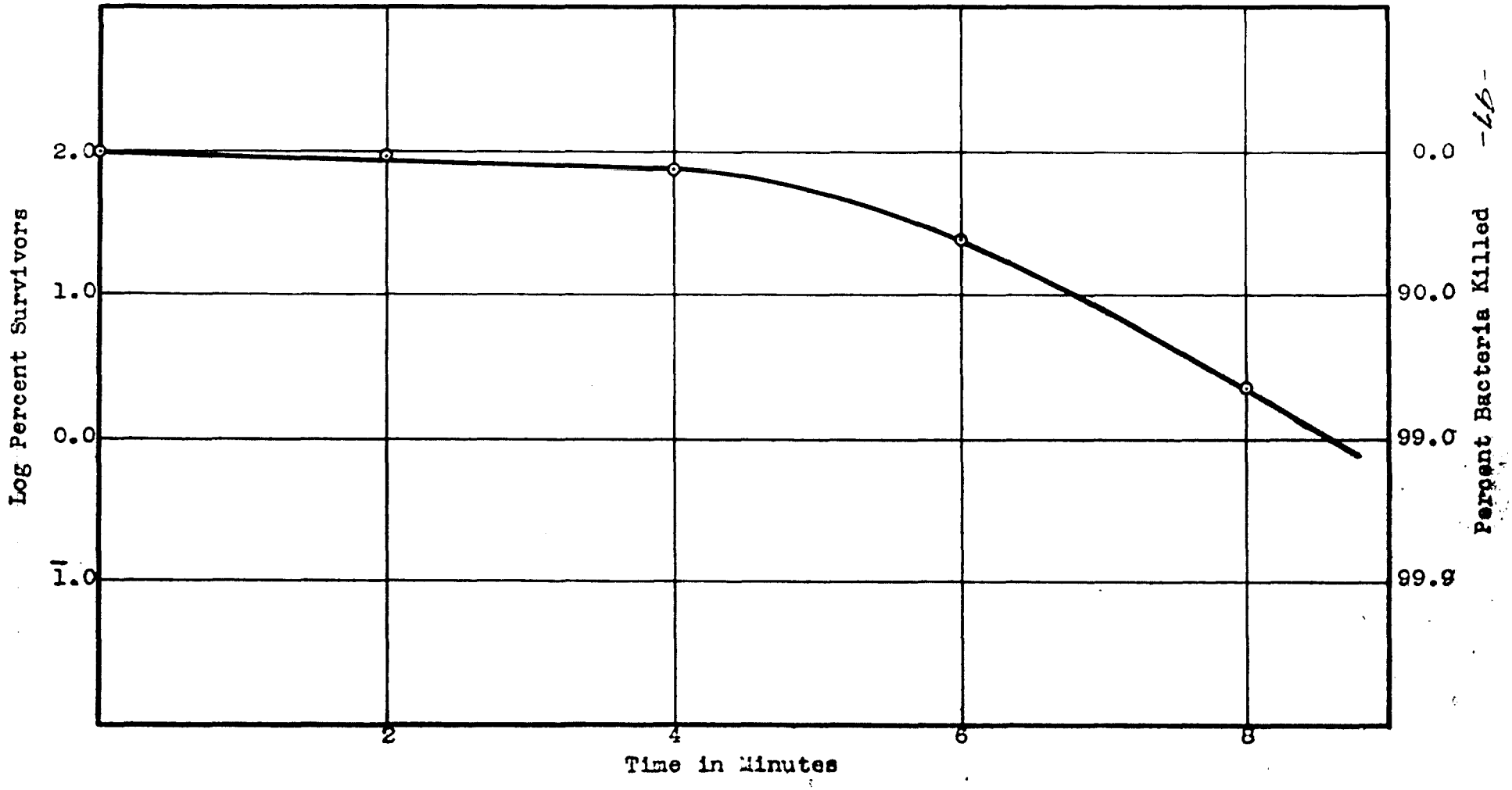


TABLE 4B

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 8.0
(25 p.p.m. Av. Cl.; 2 p.p.m. NH_3; 20° C.)

Expt. No. / Date	33 / 8/17/39	39 / 8/21/39	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *			
0	1,250	800	100	2.00
5	900	650	77	1.89
10	700	600	66	1.82
15	380	160	25	1.40
20	49	8	2.5	0.40

			Average
pH **	8.01	7.95	7.98
Res. Cl. p.p.m. **	8.2	8.1	8.2
Killing Time (min.) #	22	20	21

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%

Fig. 4B

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 8.0
(25 p.p.m. Av. Cl.; 2 p.p.m. NH_3; 20° C.)

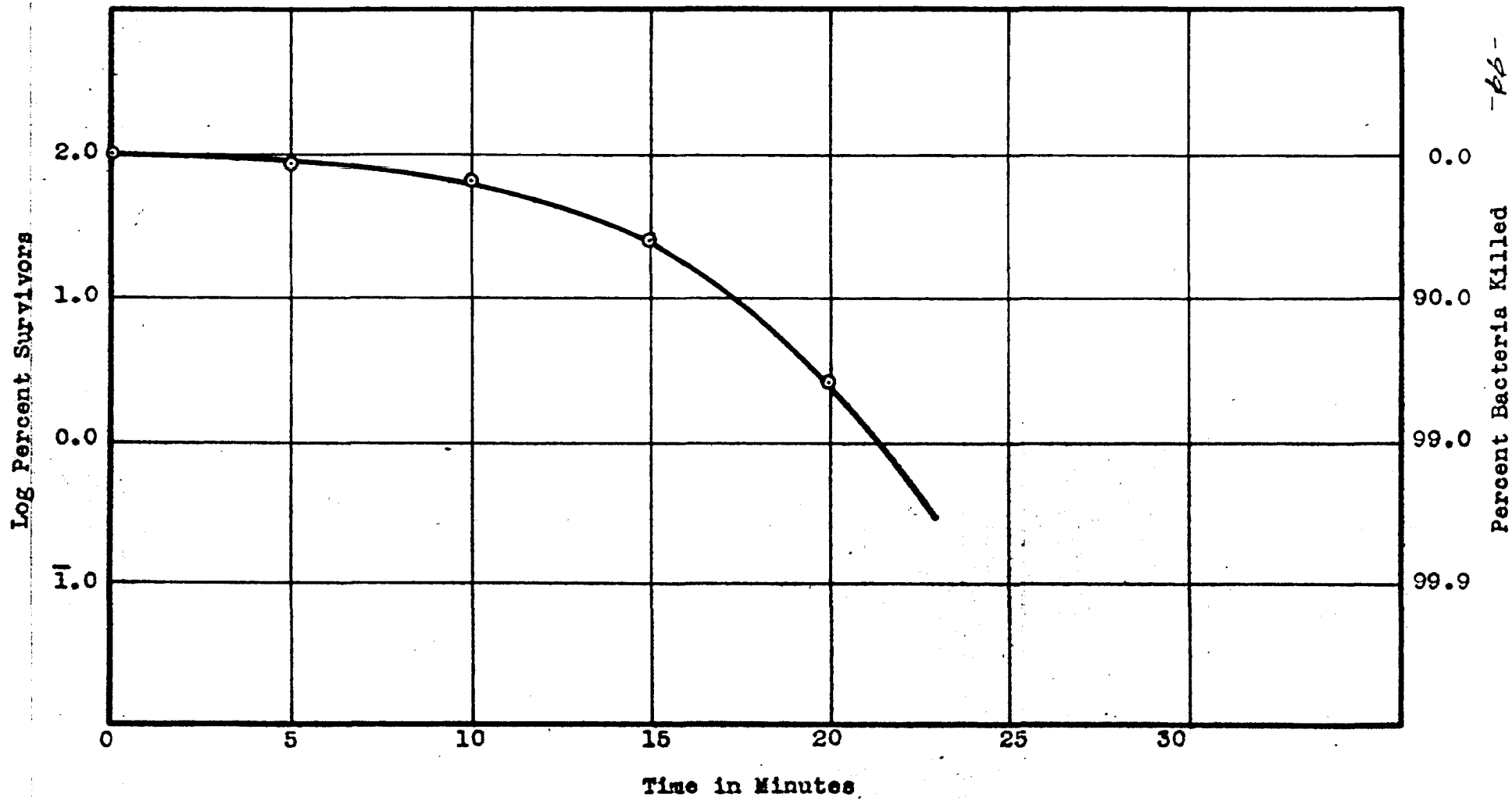


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TABLE 4C

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 8.0
(25 p.p.m. Av. Cl.; 6 p.p.m. NH_3; 20° C.)

Expt. No. / Date	20 / 8/12/39	27 / 8/17/39	37 / 8/21/39	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0	1,050	800	800	100	2.00
10	700	800	800	89	1.95
20	305	410	550	50	1.70
30	195	305	450	38	1.58
40	150	185	295	25	1.40
50	60	70	120	9.8	0.99
60	22	75	65	6.5	0.81
70	10	30	18	2.4	0.38
80	5	9.5	18.5	1.3	0.11
90	3.25 ##	9.5	8.5	0.87	$\bar{1}.94$
100		2.25	3.5		

				Average
pH **	8.02	7.99	8.00	8.00
Res. Cl. p.p.m. **	22.1	22.3	22.4	22.3
Killing Time (min.) #	73	88	89	83

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%
Estimated from survivor curve

Fig. 40

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 8.0
(25 p.p.m. Av. Cl.; 6 p.p.m. NH_3; 20° C.)

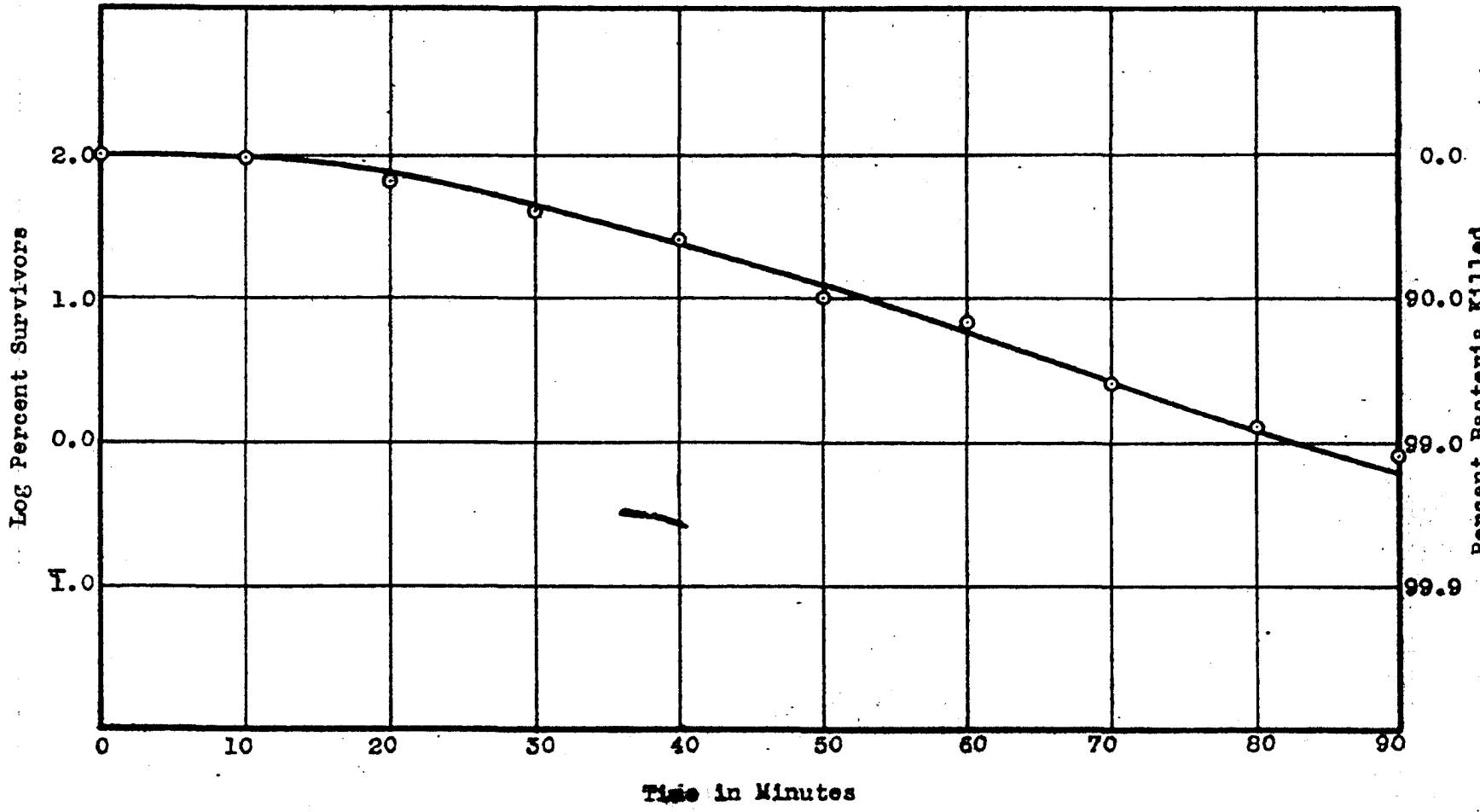

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TABLE 4D

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 8.0
(25 p.p.m. Av. Cl.; 18 p.p.m. NH_3; 20° C.)

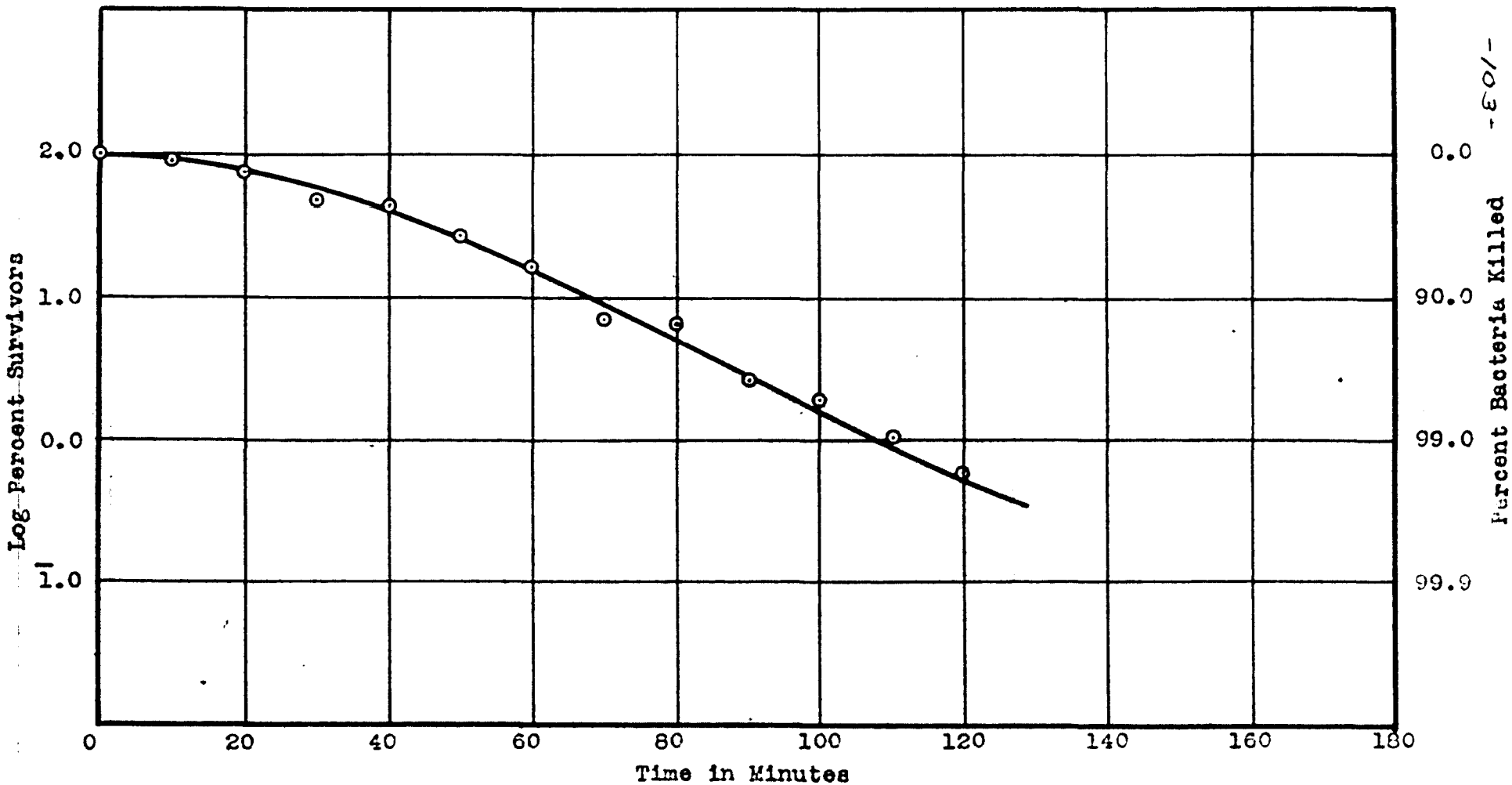
Expt. No. / Date	26 / 8/14/39	28 / 8/17/39	38 / 8/21/39	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0	950	800	800	100	2.00
10	750	600	900	89	1.95
20	550	500	700	73	1.86
30	265	425	425	45	1.65
40	230	405	400	42	1.62
50	250	100	260	24	1.38
60	145	110	170	16	1.20
70	49	55	65	6.7	0.83
80	36	31	80	6.6	0.82
90	17.5	13.5	33	2.5	0.40
100	13.5	9.5	24.5	1.9	0.28
110	8.5 ##	2.75	15.5	1.1	0.04
120	5.5	1.5	4	0.63	$\bar{1}$.80

				Average
pH **	7.99	8.00	7.97	7.99
Res. Cl. p.p.m. **	23.8	22.0	22.9	22.9
Killing Time (min.) #	107	100	116	107

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%
Estimated from survivor curve

Fig. 4D

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 8.0
(25 p.p.m. Av. Cl.; 18 p.p.m. NH_3; 20° C.)



The results detailed in Tables 4 to 4D are summarized in Table 4E, from which it will be noted that the reactions for all of the experiments were quite constant. It will be noted that as the concentration of added ammonia was increased from 0.0 to 18 p.p.m. the time to kill 99 percent of the exposed B. metiens spores also increased from 7.6 minutes in the absence of ammonia to 107 minutes when a concentration of 18 p.p.m. ammonia was added to 25 p.p.m. available chlorine. The residual available chlorine after 15 minutes (the time when the test organism was added) was 22.0 p.p.m. in the absence of ammonia and dropped to 7.5 p.p.m. available chlorine. Upon the addition of still greater concentrations of ammonia, (6 and 18 p.p.m.), however, the residual chlorine again rose to 22.0 and 22.8 p.p.m. respectively. From the summary of these results at pH 8 it may be seen that, at this reaction, residual chlorine is not a dependable measure of probable germicidal efficiency.

TABLE 4E

SUMMARY OF RESULTS (TABLES 4 TO 4D) AT pH 8.0
(25 p.p.m. Av. Cl. added; 20° C.)

NH_3 (p.p.m.) added	0.0	0.5	2	6	18
Killing* time (min.)	7.6	8.6	21	83	107
Residual# Av. Cl. (p.p.m.) after 15 min. contact (initial)	22.0	18.6	7.5	22.0	22.8
Residual#* Av. Cl. (p.p.m.) at end of experiment	22.9	18.9	8.2	22.3	22.9
pH at end of experiment	8.00	8.00	7.98	8.00	7.99
Ratio $\frac{\text{Av. Cl.}}{NH_3}$ added		50/1	12.5/1	4.2/1	1.4/1

* Average time required to kill 99 percent exposed spores
\# From Table 7A
#* For approximate times of contact see Table 7A

e. Observations at pH 9. The data for experiments at pH 9 maintained by using a $\frac{M}{20}$ carbonate buffer (see appendix) employing 25 p.p.m. chlorine with various concentrations of ammonia, (0.0, 0.5, 2, 6 and 18 p.p.m.) are detailed in Tables 5 to 5D and the curves for the average percent survivors are shown graphically in Figures 5 to 5D.

Results at pH 9 are significantly different in many respects from those previously reported, particularly for the series of pH 7, 6, and 5.

Referring to Table 5E it may be seen that the addition of 25 p.p.m. chlorine alone showed an initial concentration of 24.2 p.p.m. with a drop to 21.7 p.p.m. for the average of determinations made at the end of each experiment. Killing times (Table 5) ranged from 54 to 63 minutes with an average of 58 minutes.

On the addition of 0.5 p.p.m. ammonia to 25 p.p.m. chlorine the initial chlorine concentration was 20.3 and dropped to 17.8 p.p.m. by the time the experiments were completed. The time required to kill 99 percent of the exposed B. metiens spores varied from 63 to 70 minutes with an average of 66 minutes (Table 5A).

When a concentration of 2 p.p.m. of ammonia was added to 25 p.p.m. chlorine at pH 9 (Table 5E) the initial chlorine concentration had dropped to 11.1 and remained rather constant for the duration of the experiments. The killing

times varied from 136 to 160 minutes with an average of 150 minutes as may be seen in Table 5B.

On the addition of 6 p.p.m. ammonia to 25 p.p.m. available chlorine (the ratio required for the production of monochloro-amine) the initial chlorine was 23.0 p.p.m. while the average of residuals (at the end) had dropped to 17.9 p.p.m. Table 5C shows that the residual chlorine concentrations (at the end) for the individual experiments fluctuated somewhat more than had been previously observed and the killing time likewise varied considerably. Thus, in two experiments the killing times were 135 and 145 minutes while in five experiments the time to kill 99 percent of the exposed spores varied from 180 to 220 minutes. The average killing time was 182 minutes. The reason for this greater fluctuation (in the results obtained at pH 9) than was obtained with more acid solutions has not been ascertained, except that it could not be attributed to a change in reaction (pH) which remained quite constant.

With 18 p.p.m. of ammonia added to 25 p.p.m. chlorine at pH 9 the initial chlorine concentration was 22.6 p.p.m. with a drop to 20.2 p.p.m. during the course of the experiments, as may be seen in Table 5E. The killing time (Table 5D) varied from 243 to 285 minutes with an average of 263 minutes.

Figures 5 to 5D on which are shown graphically the average percents of survivors detailed in Table 5 to 5D show that there are distinct departures from the corresponding figures for the more acid solutions. With the more acid solutions and the lower ammonia concentrations, the curves showed a marked lag followed by increasing death rates. At pH 9, although the marked lag is still manifest, the death rate, after death begins, is much more constant, approaching a straight line. With the higher concentrations of ammonia, when employing 6 and 18 p.p.m., the lag is almost completely lacking, and the entire survivor curve approaches a straight line.

TABLE 5

RESISTANCE OF B. METIENS SPORES TO CHLORINE AT pH
(25 p.p.m. Av. Cl; 20° C.)

Expt. No.	15	85	95	99	104	108	
Date	8/8/39	10/15/39	10/28/39	11/4/39	11/11/39	11/18/39	4/
Exposure Time (in min.)	Surviving Bacteria in Thousands *						
0	1,150	900	650	950	850	1,050	1
10	1,250	1,150	500	1,150	950	950	1
20	750	500	550	1,000	1,150	1,100	1
30	850	600	230	500	900	800	1
40	130	170	38.5	115	115	295	
50	25	60	14	18.5	35	40	
60	11	12.5	2.25	6	13.5	20.5	
70		1.75		1.25	2.25		
pH **	9.03	9.03	9.00	8.98	8.98	9.01	
Res. Cl. p.p.m. **	23.0	21.8	22.0	21.3	20.6	20.7	
Killing Time (min.) #	57	63	54	56	62	61	

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%

TABLE 5

SISTANCE OF B. METIENS SPORES TO CHLORINE AT pH 9.0
(25 p.p.m. Av. Cl; 20° C.)

5 : 95 : 99 : 104 : 108 : 187 : Average : Log Average
5/39: 10/28/39 : 11/4/39: 11/11/39 :11/18/39 : 4/22/40: % : %
Surviving Bacteria in Thousands * : Survivors: Survivors
00 650 950 850 1,050 1,100 100 2.00
50 500 1,150 950 950 1,000 104 2.02
00 550 1,000 1,150 1,100 1,150 93 1.97
00 230 500 900 800 1,000 72 1.86
70 38.5 115 115 295 125 15 1.18
60 14 18.5 35 40 22.5 3.3 0.52
12.5 2.25 6 13.5 20.5 7.5 1.1 0.04
1.75 1.25 2.25

						Average
).03	9.00	8.98	8.98	9.01	9.03	9.01
..8	22.0	21.3	20.6	20.7	22.4	21.7
)	54	56	62	61	56	58

housands) per 5 ml.

RATE OF DEATH OF *E. COLI* STRAIN 25 ($\frac{25}{25}$) EXPOSED TO CHLORINE AT pH 9.0 AND 20° C.)

Fig. 5

TABLE 5A

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 9.0
(25 p.p.m. Av. Cl.; 0.5 p.p.m. NH_3; 20° C.)

Expt. No. / Date	94 / 10/28/39	98 / 11/4/39	103 / 11/11/39	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0	650	950	850	100	2.00
10	500	1,100	800	96	1.98
20	700	1,350	1,300	130	2.11
30	455	600	445	65	1.81
40	130	270	420	32	1.51
50	30	60	105	7.6	0.88
60	11	22	27	2.4	0.38
70	2.5	13.5	14.5	1.2	0.08
80		1.5	3.25		

				Average
pH **	9.03	8.97	9.01	9.00
Res. Cl. p.p.m. **	18.2	17.5	17.6	17.8
Killing Time (min.) #	63	66	70	66

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%

Fig. 5A

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 9.0
(25 p.p.m. Av. Cl.; 0.5 p.p.m. NH_3; 20° C.)

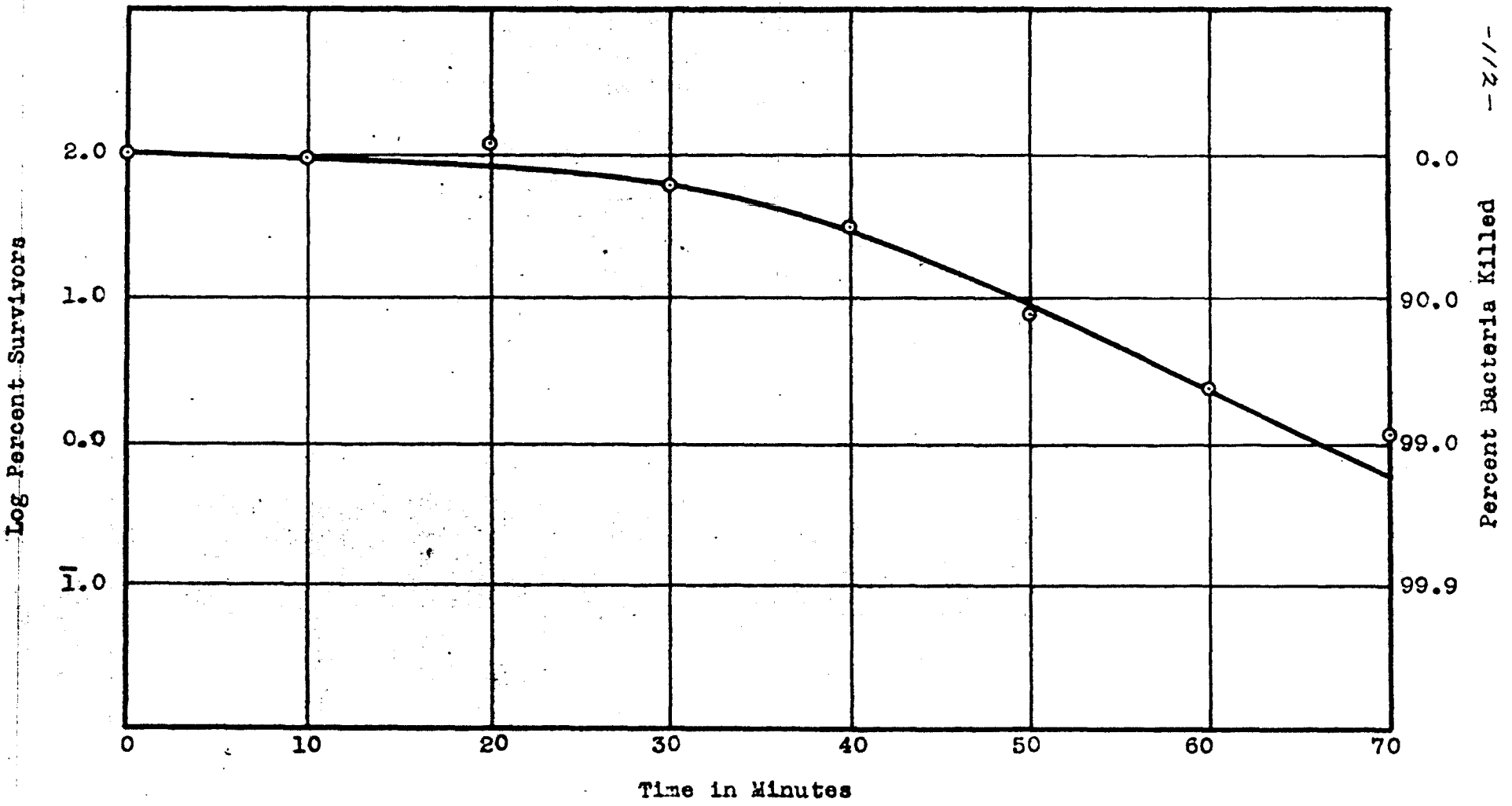


TABLE 5B

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 9.0
(25 p.p.m. Av. Cl.; 2 p.p.m. NH_3; 20° C.)

Expt. No. / Date	97 / 10/28/39	102 / 11/4/39	107 / 11/11/39	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0	650	950	850	100	2.00
20	500	1,150	950	103	2.01
40	425	800	900	85	1.93
60	450	800	800	82	1.91
80	165	650	700	59	1.77
100	42.5	215	175	17	1.23
120	17	44	49	4.3	0.63
140	5.5	18.5	33	2.2	0.34
160	1.25 ##	10.5	11	0.90	$\bar{1}$.95
180		2.25	5.5		

				Average
pH **	9.03	8.99	9.00	9.01
Res. Cl. p.p.m. **	8.2	7.5	7.7	7.8
Killing Time (min.) #	136	155	160	150

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%
Estimated from survivor curve

Fig. 5B

RESISTANCE OF *B. METIENS* SPORES TO CHLORINE AND AMMONIA AT pH 9.0
(25 p.p.m. Av. Cl.; 2 p.p.m. NH_3; 20° C.)

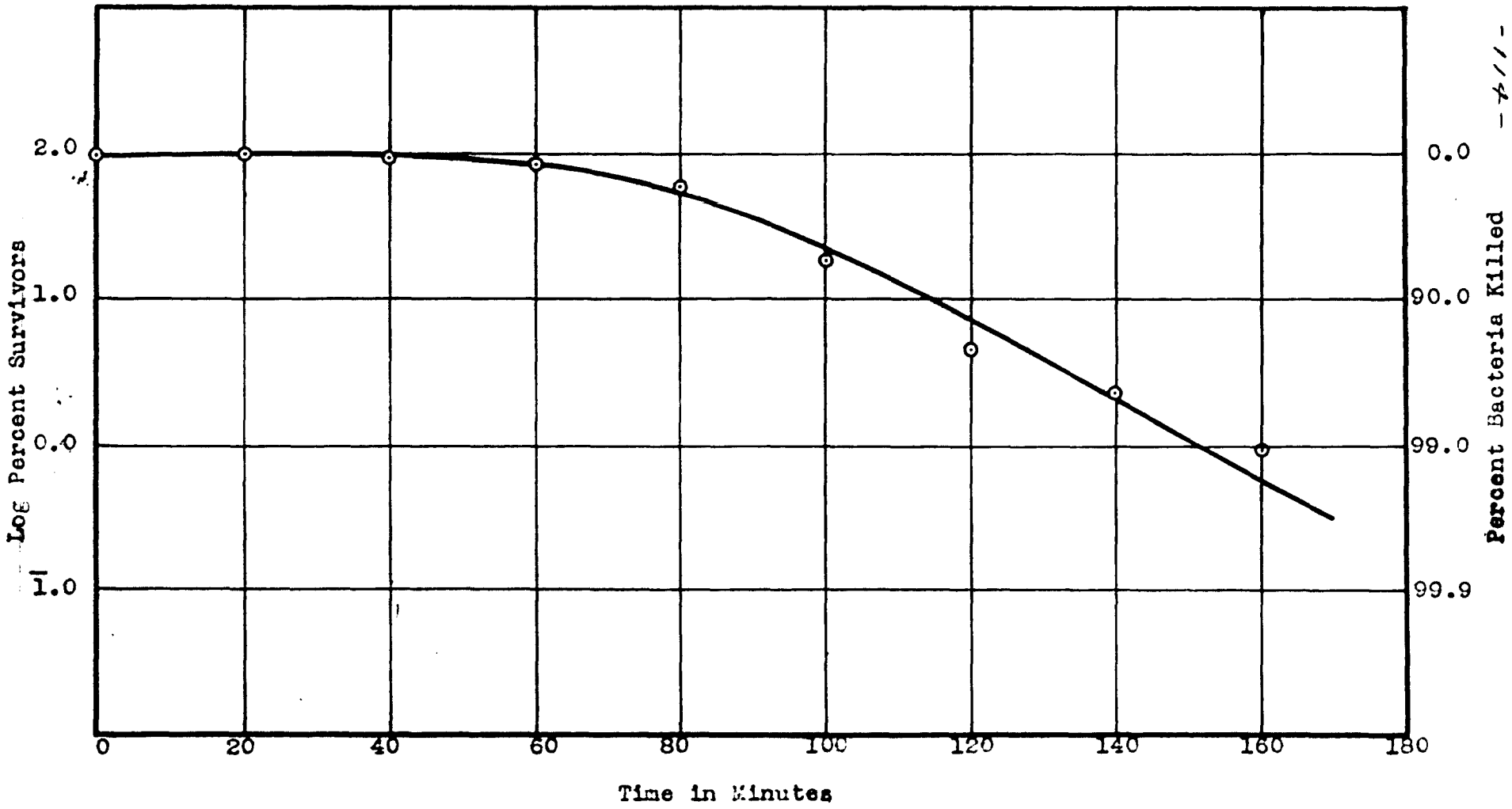


TABLE 5C

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA
(25 p.p.m. Av. Cl.; 6 p.p.m. NH_3; 20° C.)

Expt. No.	96	101	106	111	113	155	
Date	10/28/39	11/4/39	11/11/39	11/25/39	11/26/39	3/2/40	3
Exposure Time (in min.)	Surviving Bacteria in Thousands *						
0	650	950	850	750	900	1,700	1
20	370	395	650	700	650	1,100	
40	190	235	270	700	490	550	
60	80	155	200	255	235	325	
80	32	130	60	210	135	155	
100	17	80	30.5	85	120	140	
120	15.5	41.5	22.5	90	55	75	
140	5.5	17.5	16	30	30	23	
160	4.4	19	15	31	16.5	7.5	
180	2.4	8.5	16	12	9	5	
200	2.2	7	11	9	9.5	4.5	
220		3	8	6	8		
pH **	9.00	9.00	9.03	9.04	9.04	9.01	
Res. Cl. p.p.m. **	20.2	16.1	17.4	16.8	17.7	18.7	1
Killing Time (min.) #	135	180	220	200	194	145	2(

* Surviving bacteria (in thousands) per 5 ml.
** At the end of experiment
\# Time for killing 99%

TABLE 5C

OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 9.0
(25 p.p.m. Av. Cl.; 6 p.p.m. NH_3; 20° C.)

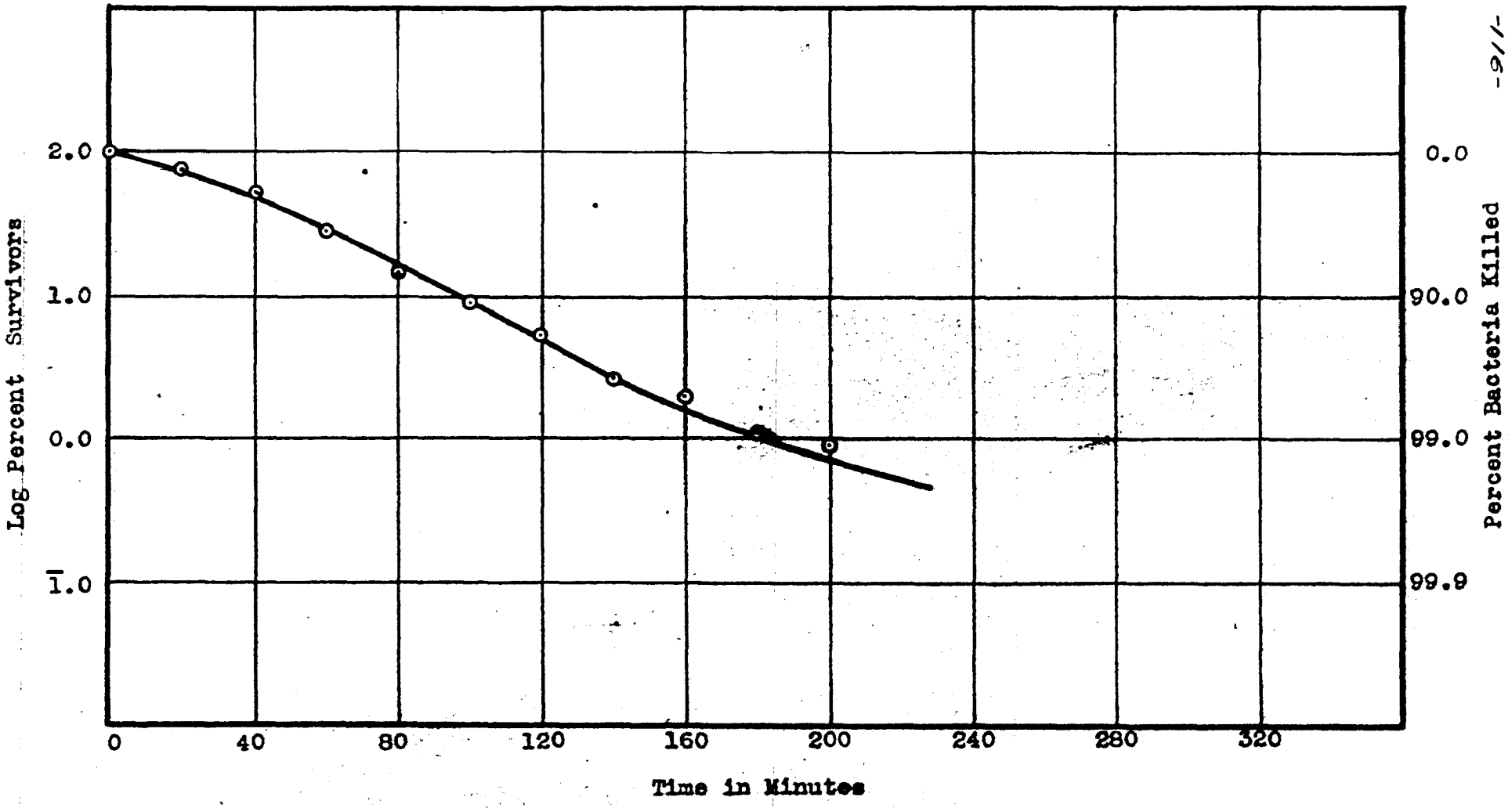
106 : 11/11/39	111 : 11/25/39	113 : 11/26/39	155 : 3/2/40	157 : 3/9/40	Average % Survivors	Log Average % Survivors
Surviving Bacteria in Thousands *						
850	750	900	1,700	1,000	100	2.00
650	700	650	1,100	900	71	1.85
270	700	490	550	550	46	1.66
200	255	235	325	600	27	1.43
60	210	135	155	175	14	1.15
30.5	85	120	140	150	8.8	0.94
5 22.5	90	55	75	65	5.4	0.73
5 16	30	30	23	44	2.5	0.40
15	31	16.5	7.5	21	1.9	0.28
5 16	12	9	5	16.5	1.1	$\bar{0}.04$
11	9	9.5	4.5	13.5	0.91	$\bar{1}.96$
8	6	8		7.5		

					Average
) 9.03	9.04	9.04	9.01	9.00	9.02
17.4	16.8	17.7	18.7	18.2	17.9
220	200	194	145	200	182

isands) per 5 ml.

Fig. 5C

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 9.0
(25 p.p.m. Av. Cl.; 6 p.p.m. NH_3; 20° C.)



-9/-

TABLE 5D

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 9.0
(25 p.p.m. Av. Cl.; 18 p.p.m. NH_3; 20° C.)

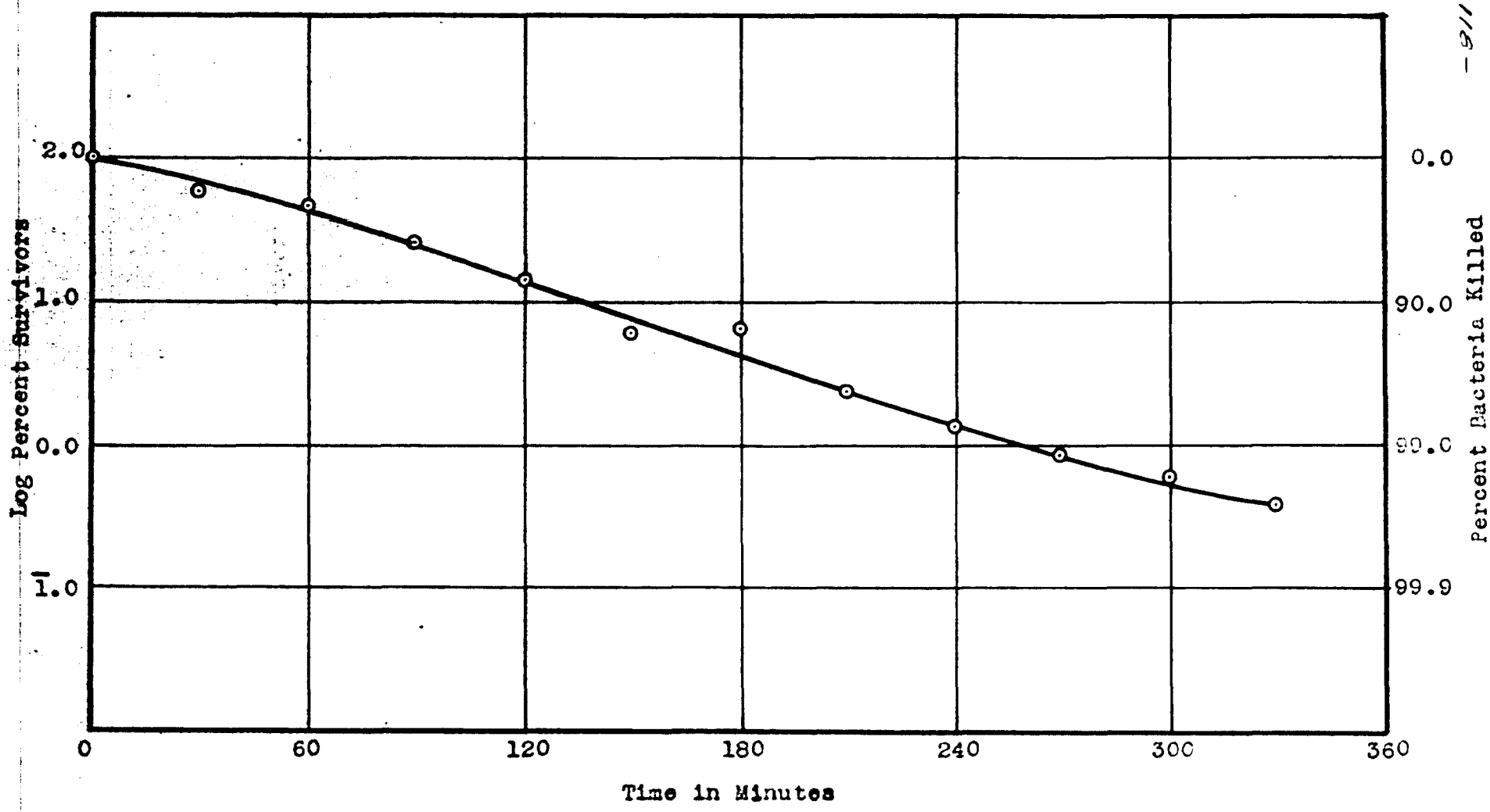
Expt. No. / Date	93 / 10/28/39	100 / 11/4/39	105	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0	650	950	850	100	2.00
30	370	480	550	58	1.76
60	335	325	450	46	1.66
90	155	200	250	25	1.40
120	70	135	140	14	1.15
150	35.5	80	33	5.9	0.77
180	23	42	100	6.6	0.82
210	12.5	16	29	2.3	0.36
240	6.5	12.5	13	1.3	0.11
270	4.25	8	10.5	0.90	$\bar{1}$.95
300	3	1.75	10	0.61	$\bar{1}$.79
330	1.75	2.25	5.5	0.38	$\bar{1}$.58

				Average
pH **	9.00	8.99	9.00	9.00
Res. Cl. p.p.m. **	21.0	20.1	19.3	20.2
Killing Time (min.) #	243	261	285	263

* Surviving bacteria (in thousands) per 5 ml.
** At the end of experiment
\# Time for killing 99%

Fig. 5D

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 9.0
(25 p.p.m. Av. Cl.; 18 p.p.m. NH_3; 20° C.)



The data from Tables 5 to 5D are summarized in Table 5E for pH 9. Attention is called to the fact that the reaction remained constant for all of the experiments, fluctuating only between pH 9.00 and 9.02.

At the end of 15 minutes (the time at which the spores were added) the residual chlorine decreased from 24.2 p.p.m. in the absence of ammonia to 20.3 on the addition of 0.5 p.p.m. and 11.1 on the addition of 2 p.p.m. ammonia. Thereafter it increased to 23.0 p.p.m. available chlorine in the presence of 6 p.p.m. ammonia and to 22.6 when 18 p.p.m. ammonia had been added. The killing times, in a manner analogous to what was observed for pH 8, progressively increased with increasing concentrations of ammonia from 58 minutes in the absence of ammonia, to 66 minutes when employing 0.5 p.p.m. ammonia, 150 minutes upon the addition of 2 p.p.m. of ammonia, 182 minutes with 6 p.p.m. of ammonia and 263 minutes when 18 p.p.m. of ammonia had been added to 25 p.p.m. of chlorine. Here again it will be noted that the residual chlorine was not a dependable measure of the germicidal efficiency.

TABLE 5E

SUMMARY OF RESULTS (TABLES 5 TO 5D) AT pH 9.0
(25 p.p.m. Av. Cl. added; 20° C.)

NH_3 (p.p.m.) added	0.0	0.5	2	6	18
Killing* time (min.)	58	66	150	182	263
Residual# Av. Cl. (p.p.m.) after 15 min. contact (initial)	24.2	20.3	11.1	23.0	22.6
Residual#* Av. Cl. (p.p.m.) at end of experiment	21.7	17.8	7.8	17.9	20.2
pH at end of experiment	9.01	9.00	9.01	9.02	9.00
Ratio $\frac{\text{Av. Cl. added}}{NH_3}$		50/1	12.5/1	4.2/1	1.4/1

* Average time required to kill 99 percent exposed spores
\# From Table 7A
#* For approximate times of contact see Table 7A

f. Observations at pH 10. The results obtained at pH 10 (buffered with $\frac{M}{20}$ carbonate buffer (see appendix), are detailed in Tables 6 to 6D. The germicidal efficiencies were extremely low in the absence of ammonia or in the presence of small quantities of ammonia and this was also the case when dealing with excessive quantities of ammonia as will be observed below.

At pH 10, when 25 p.p.m. available chlorine had been added, with no ammonia present, the initial chlorine concentration was 24.2 p.p.m. (Table 6E). This initial concentration dropped during the course of the experiments so that the average of the residuals determined at the end of each experiment was 20.3 p.p.m. The killing times were 558 and 582 minutes or an average of 570 minutes (Table 6). This is approximately ten times as great a killing time as that obtained at pH 9.

On the addition of 0.5 p.p.m. ammonia the initial chlorine was 21.1 p.p.m. and the residual (average for determinations made at the end of each experiment) was 16.8 p.p.m. The times required to kill 99 percent of the exposed spores varies from 624 to 678 minutes with an average of 660 minutes, again, ten times that observed at pH 9.

At pH 10, employing 25 p.p.m. available chlorine and 2 p.p.m. ammonia the initial chlorine concentration dropped to 13.3 p.p.m. The chlorine concentration dropped further

during the course of the experiments, to 8.1 p.p.m. (at the end). The killing time was so prolonged that 99 percent of the spores were not killed even after 12 hours exposure.

With 6 p.p.m. of ammonia (giving a ratio of available chlorine to ammonia of 4.2 to 1 which is approximately the theoretical ratio required for the production of monochloro-amine) the initial chlorine was 23.0 p.p.m. and dropped to an average of 20.6 p.p.m. during the course of the experiments (Table 6E). The killing times varied from 180 to 198 minutes or an average of 186 minutes (Table 6C). This killing time is almost the same as that obtained at pH 9 when employing the same concentration of chlorine and ammonia.

When a concentration of 18 p.p.m. ammonia was employed with 25 p.p.m. available chlorine the initial chlorine concentration was 23.0 p.p.m. The average of residuals determined at the end of each experiment was 21.0 p.p.m. The times required to kill 99 percent of the exposed spores varied from 450 to 462 minutes with an average of 456 minutes although the initial chlorine was the same as that present at pH 10 when employing 6 p.p.m. ammonia, in which case a killing time of 186 minutes was obtained.

The shapes of the curves for the average percents of survivors shown in Table 6 to 6D and indicated graphically in Figure 6 to 6D were similar to those obtained at pH 9,

except that in the experiments employing 2 p.p.m. ammonia to 25 p.p.m. available chlorine at pH 10, the rate of death was so low that the survivor curve shown in Figure 65 did not go beyond the lag phase even after an exposure period of 12 hours.

TABLE 6

RESISTANCE OF B. METIENS SPORES TO CHLORINE AT pH 10.0
(25 p.p.m. Av. Cl.; 20° C.)

Expt. No. / Date	130 / 1/13/40	135 / 1/20/40	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *			
0	1,100	650	100	2.00
60	1,150	550	95	1.98
120	1,150	850	120	2.08
180	850	700	94	1.97
240	900	480	78	1.89
300	700	365	60	1.78
360	375	155	29	1.46
420	65	50	6.8	0.83
480	33.5	23	3.3	0.52
540	21.5	11	1.9	0.28
600	6	6	0.74	$\bar{1}$.87
660		2		

			Average
pH **	9.99	9.97	9.98
Res. Cl. p.p.m. **	20.2	20.4	20.3
Killing Time (min.) #	558	582	570

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%

Fig. 6

RESISTANCE OF B. METIENS SPORES TO CHLORINE AT pH 10.0
(25 p.p.m. Av. Cl.; 20° C.)

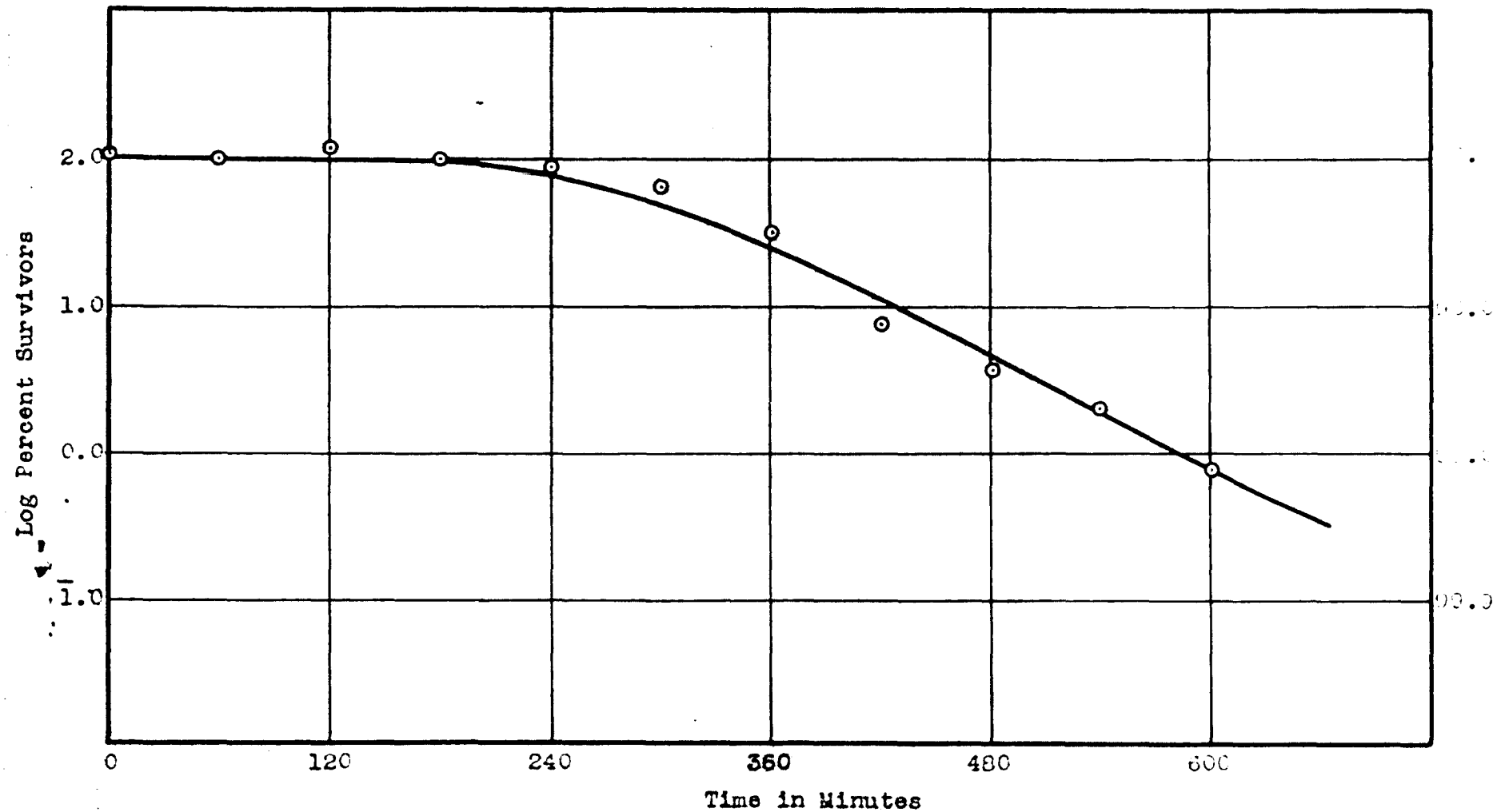


TABLE 6A

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 10.0
(25 p.p.m. Av. Cl.; 0.5 p.p.m. NH_3; 20° C.)

Expt. No. / Date	131 / 1/14/40	136 / 1/21/40	137 / 1/21/40	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0	800	950	600	100	2.00
60	---	1,000	550	---	----
120	650	700	650	88	1.94
180	---	1,100	550	---	----
240	750	355	600	77	1.89
300	---	800	460	---	----
360	385	550	450	60	1.78
420	---	115	175	---	----
480	47.5	85	70	8.9	0.95
540	---	24	37	---	----
600	7	17	17	1.8	0.26
660	---	12.5	10.5	---	----
720	2.8	4.5	3.5	0.46	$\bar{1}$.66

				Average
pH **	9.99	10.00	10.00	10.00
Res. Cl. p.p.m. **	17.1	16.6	16.8	16.8
Killing Time (min.) #	624	678	678	660

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%

Fig. 6A

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 10.0
(25 p.p.m. Av. Cl.; 0.5 p.p.m. NH_3; 20° C.)

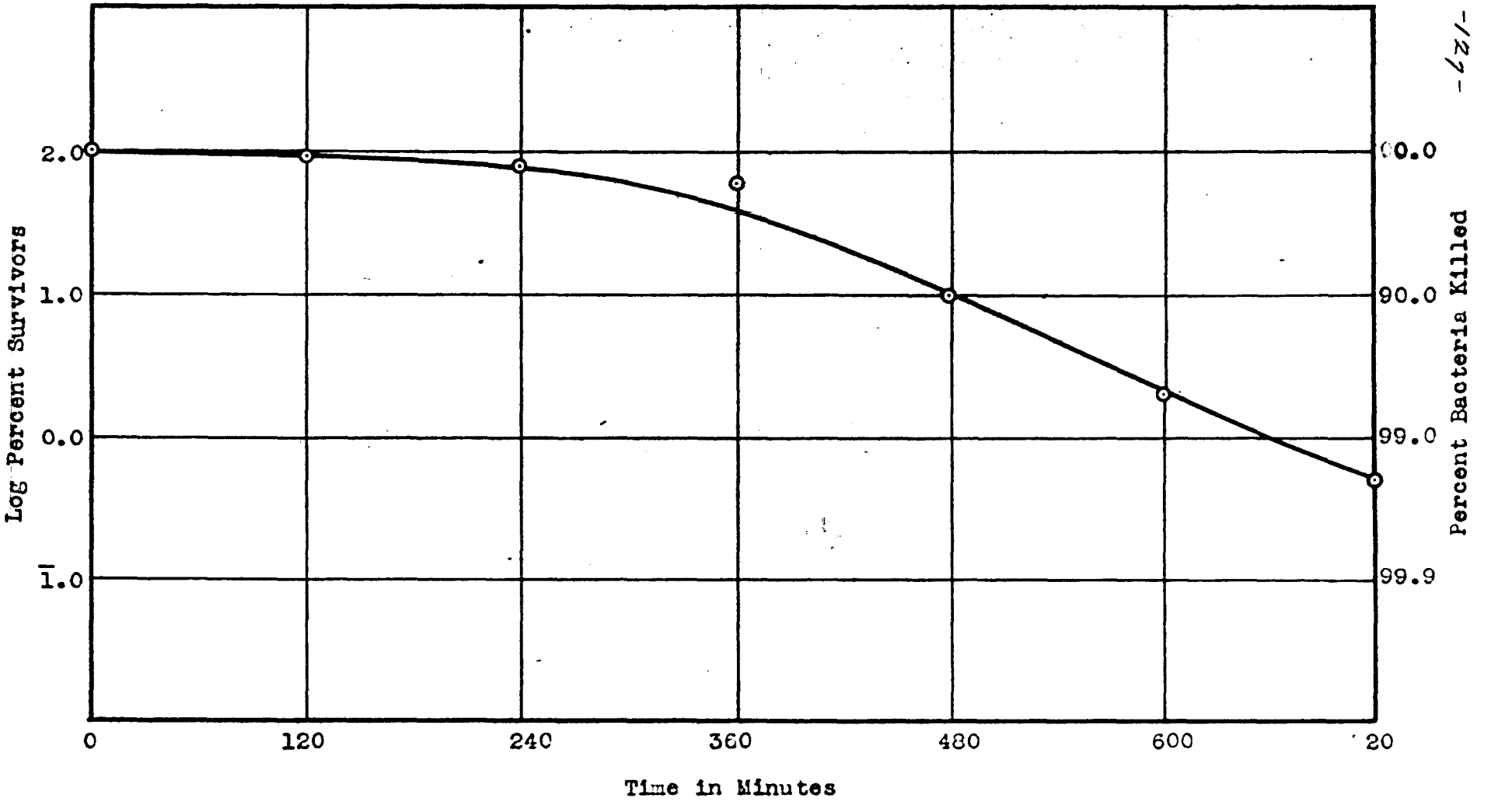


TABLE 6B

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 10.0
(25 p.p.m. Av. Cl.; 2 p.p.m. NH_3; 20° C.)

Expt. No. / Date	125 / 1/6/40	132 / 1/14/40	138 / 1/21/40	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0	850	850	650	100	2.00
120	700	900	600	93	1.97
240	1,050	800	650	110	2.04
360	650	650	---	77	1.89
480	800	750	550	89	1.95
600	600	700	600	82	1.91
720	480	650	900	91	1.96

				Average
pH **	9.97	9.97	10.00	9.98
Res. Cl. p.p.m. **	7.9	8.5	8.0	8.1
Killing Time (min.) #	##	##	##	##

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%
Not reached in 12 hours

Fig. 6B

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 10.0
(25 p.p.m. Av. Cl.; 2 p.p.m. NH_3; 20° C.)

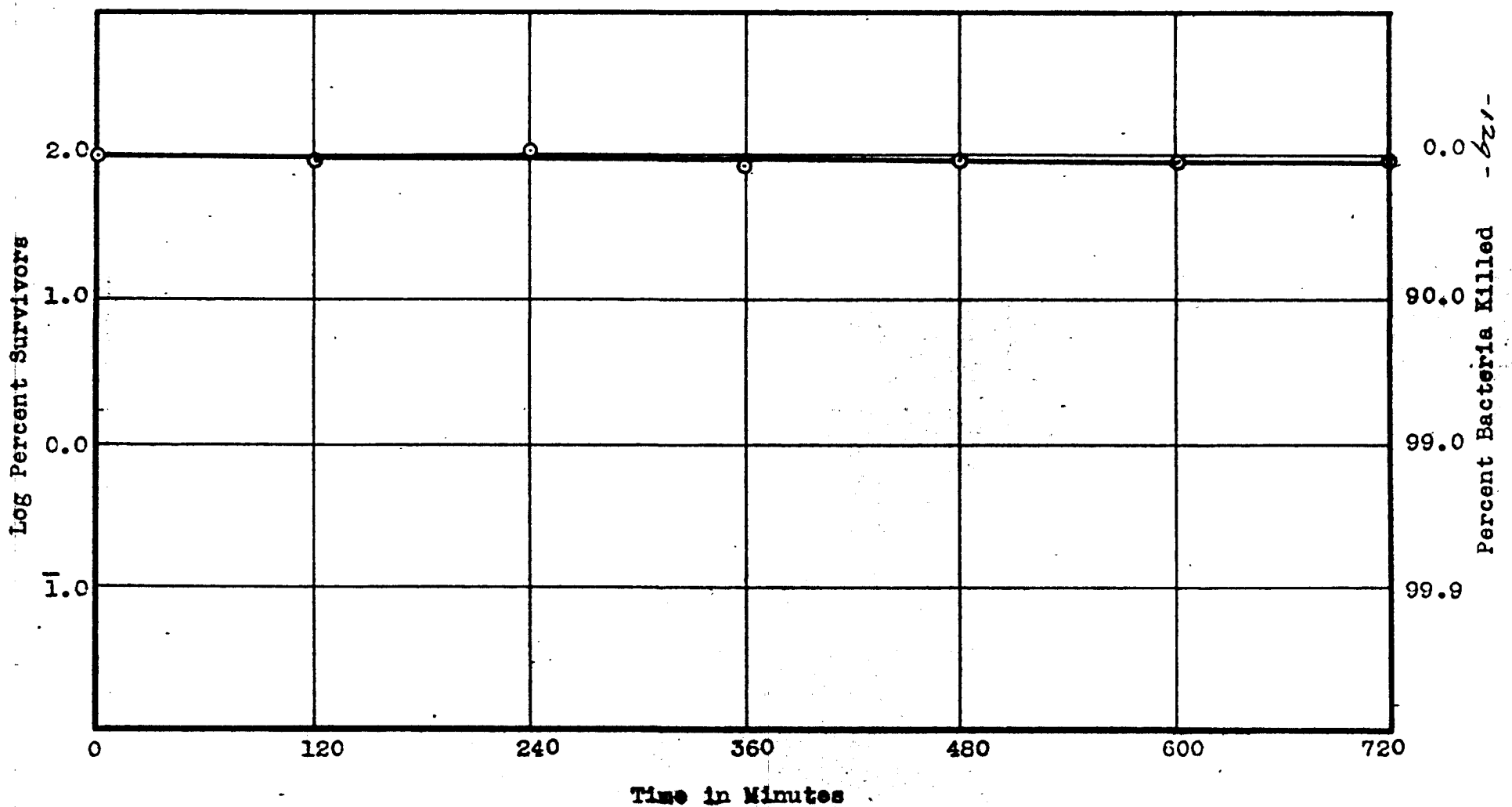


TABLE 6C

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 10.0
(25 p.p.m. Av. Cl.; 6 p.p.m. NH_3; 20° C.)

Expt. No. / Date	129 / 1/13/40	134 / 1/20/40	156 / 3/2/40	Average % Survivors	Log Average % Survivors
Exposure Time (in min.)	Surviving Bacteria in Thousands *				
0	850	900	1,450	100	2.00
30	550	350	800	53	1.72
60	205	200	350	23	1.36
90	90	95	205	12	1.08
120	75	36.5	49.5	5.4	0.73
150	12.5	19	29.5	1.9	0.29
180	14.5	9	6	1.0	0.00
210	6.5	3.75	3.7	0.48	$\bar{1}$.68
240	9.5	6.5			
270	5	1.2			

				Average
pH **	10.01	9.97	10.00	9.99
Res. Cl. p.p.m. **	19.9	20.7	21.1	20.6
Killing Time (min.) #	198	186	180	186

* Surviving bacteria (in thousands) per ml.
** At end of experiment
\# Time for killing 99%

Fig. 6C

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 10.0
(25 p.p.m. Av. Cl.; 6 p.p.m. NH_3; 20° C.)

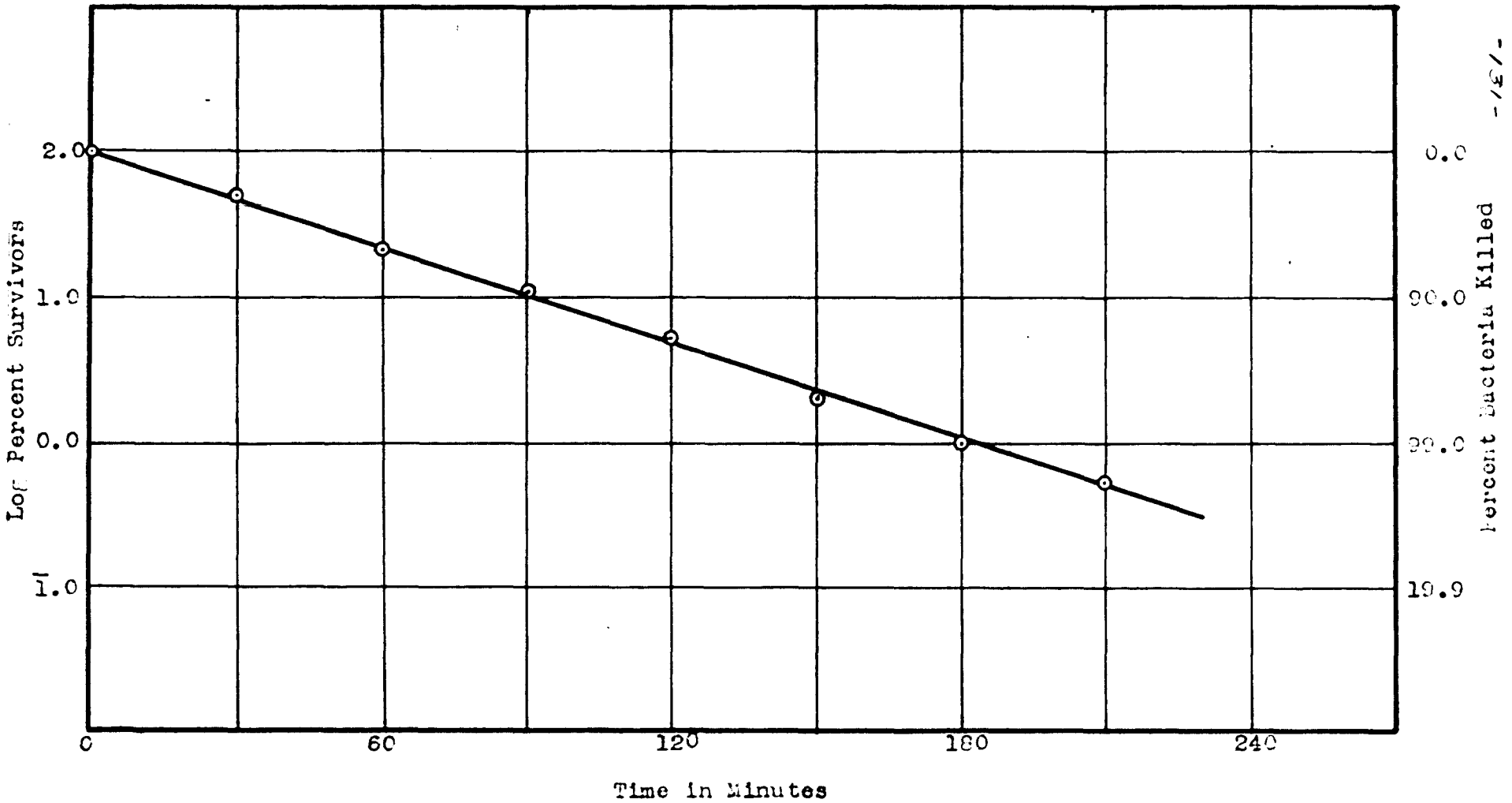


TABLE 6D

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 10.0
(25 p.p.m. Av. Cl.; 18 p.p.m. NH_3; 20° C.)

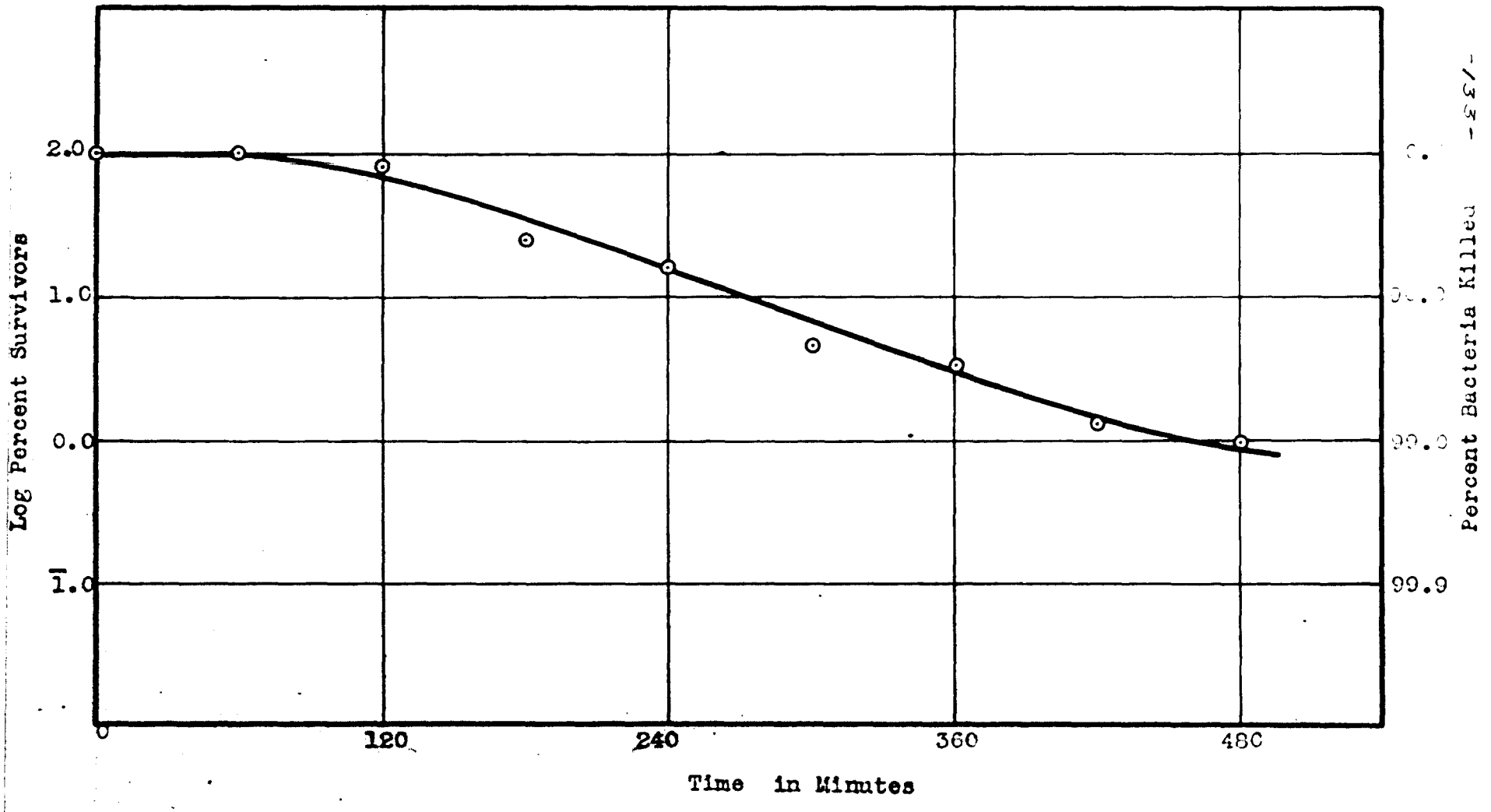
Expt. No.	128	133	Average	Log Average
Date	1/13/40	1/20/40	%	%
Exposure Time (in min.)	Surviving Bacteria in Thousands *		Survivors	Survivors
0	900	750	100	2.00
60	900	700	97	1.99
120	470	750	76	1.88
180	180	195	23	1.36
240	145	105	15	1.18
300	55	18	4.3	0.63
360	30	25	3.3	0.52
420	5.5	14	1.3	0.11
480	8	6.5	0.88	$\bar{1}.94$

			Average
pH **	9.97	9.97	9.97
Res. Cl. p.p.m. **	21.3	20.7	21.0
Killing Time (min.) #	450	462	456

* Surviving bacteria (in thousands) per 5 ml.
** At end of experiment
\# Time for killing 99%

Fig. 6D

RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 10.0
(25 p.p.m. Av. Cl.; 18 p.p.m. NH_3; 20° C.)



The data detailed in Table 6 to 6D at pH 10 are summarized in Table 6E. Attention is called first to the fact that the reaction was quite constant, fluctuating between pH 9.97 and 10.00. The initial chlorine residual after 5 minutes, at which time the B. metiens spores were added, dropped to a minimum of 13.3 p.p.m. when 2 p.p.m. ammonia had been added. Smaller quantities of ammonia 0.5 p.p.m. showed an initial concentration of 21.1 p.p.m. whereas the series in which no ammonia had been added gave an initial concentration of 24.2 p.p.m. When employing both 6 and 18 p.p.m. ammonia the initial chlorine was 23.0 p.p.m. in both cases. The killing times were extremely high, particularly with the solutions in which the ratio of available chlorine to ammonia (added) was 12.5 to 1 or greater.

In general, the killing times at pH 10 were ten times those for the same concentrations of chlorine and ammonia at pH 9. Thus a killing time of 58 minutes at pH 9 rose to 570 minutes at pH 10 when 25 p.p.m. of chlorine was employed in the absence of ammonia. The initial chlorine concentrations were 24.2 p.p.m. in both cases. On the addition of 0.5 p.p.m. of ammonia the initial chlorine at pH 9 and 10 were 20.3 and 21.1 respectively but the killing times were 66 and 660 minutes (again a ratio of 10 to 1). With 2 p.p.m. of ammonia the initial chlorine at pH 9 was 11.1 p.p.m. as compared to 13.3 p.p.m. at pH 10 and the killing time was 150

minutes at pH 9 whereas at pH 10 less than 20 percent of the bacteria had been killed in 12 hours. Should this ratio of 10 to 1 hold it would have required about 25 hours to kill 99 percent of the spores at pH 10 when 2 p.p.m. of ammonia had been added to 25 p.p.m. The picture is quite different when comparing the data at pH 9 and 10 employing higher concentrations of ammonia. There was no appreciable difference in the killing time at pH 9 and 10 when employing 6 p.p.m. ammonia added to 25 p.p.m. of chlorine, the respective times being 182 and 186 minutes. The initial chlorine concentrations were the same at both reactions, being 23.0 p.p.m. On increasing the ammonia content to 18 p.p.m., however, the decreased rate of germicidal effeciency, as indicated by an increased killing time, was considerably greater at pH 10 than at pH 9, thus, at pH 9 the killing time was 263 minutes, whereas at pH 10 it rose to 456 minutes. The residual chlorine at pH 10 as was observed at other reactions was not a dependable index of germicidal efficiency.

TABLE 6E

SUMMARY OF RESULTS (TABLES 6 TO 6D) AT pH 10
(25 p.p.m. Av. Cl. added; 20° C.)

NH_3 (p.p.m.) added	0.0	0.5	2	6	18
Killing* time (min.)	570	660	**	186	456
Residual# Av. Cl. (p.p.m.) after 15 min. contact (initial)	24.2	21.1	13.3	23.0	23.0
Residual#* Av. Cl. (p.p.m.) at end of experiment	20.3	16.8	8.1	20.6	21.0
pH at end of experiment	9.98	10.00	9.98	9.99	9.97
Ratio $\frac{\text{Av. Cl. added}}{NH_3}$		50/1	12.5/1	4.2/1	1.4/1

* Average time required to kill 99 percent exposed spores
\# From Table 7A
#* For approximate times of contact see Table 7A
** Killing time not reached in 12 hours

g. Resume and Discussion. In some collateral studies (Weber, Bender and Levine, 1940) made since the accumulation of the data presented herein, it has been demonstrated that when chlorine is added to ammonia in a ratio of about 7 to 1 or more, the ammonia is oxidized, probably to free nitrogen. Approximately seven parts of available chlorine disappear from solution for every part of ammonia added; the excess chlorine remaining presumably exists in the form of hypochlorous acid or, in alkaline solutions, as hypochlorite. With ratios of available chlorine to ammonia less than 7 to 1 chloramines are formed. Therefore in the experiments previously described (Tables 1E to 6E) when 2 p.p.m. of ammonia or less was employed the residual chlorine consisted of hypochlorites, whereas with larger quantities of ammonia (6 and 18 p.p.m. added to 25 p.p.m. of chlorine) the residuals consisted of chloramines. The results may therefore be taken as indicative of the germicidal efficiency of hypochlorites and of chloramines.

In Tables 7 and 7A are summarized the data for the killing times and the residual chlorine concentrations presented in Tables 1E to 6E. In Figure 7 the killing time in minutes is plotted against the reaction (pH) for the data detailed in Table 7, and in Figure 7A the logarithm of the killing time is plotted against the reaction (pH).

Referring to Table 7A it will be noted that chlorine residuals are reported after 15 minutes, 60 minutes and at the end of the experiment. The residuals after 15 and 60 minutes were determined on solutions which did not contain the test organism while the residuals at the end of the experiments were from solutions to which the spores were exposed. The residual chlorine 15 minutes after the solutions were compounded was the initial chlorine concentration to which the test organism was exposed, since the B. metiens spores were added at this time. In the absence of ammonia (Series I) the initial concentration of chlorine at the various reactions was not markedly different. For example, at pH 5, the initial concentration was 24.2 p.p.m.; at pH 6, 23.4 p.p.m.; at pH 7, 23.0; at pH 8, 22.0 p.p.m.; but at pH 9 and 10 it had risen again to 24.2 p.p.m. It is evident that at a slightly alkaline reaction (pH 8) the chlorine residual was at a minimum (22 p.p.m.). When employing 0.5 and 2 p.p.m. of ammonia (Series II and III) the residual chlorine was also at a minimum at pH 8. The initial concentrations, however, were distinctly lower, being 18.6 p.p.m. in Series II and 7.5 p.p.m. in Series III. This decrease in the initial concentration, as was previously pointed out, was due to loss of chlorine as a result of the interaction with the ammonia which had been added.

In Series IV and V the residual chlorine consisted of chloramines, and there were no significant differences

observed in the available chlorine at the various reactions (pH 5 to pH 10). From these results, it appears that the rate of reaction between chlorine and ammonia, (when the chlorine is present in large excess) is greatest at pH 8. These findings are similar to those reported for the reaction of chlorine with glycine by Norman (1936), who states, "....the reaction is most rapid in the region of pH 7-9."

At each reaction (pH) the initial chlorine (determined after 15 minutes) was high when no ammonia was added, slightly lower when 0.5 p.p.m. had been added and reached a minimum with the addition of 2 p.p.m. ammonia. When more ammonia (6 and 18 p.p.m.) was employed, the residual again rose to values approaching those obtained in the absence of ammonia.

When concentrations of ammonia less than 2 p.p.m. were added, the amount of chlorine lost was approximately 7 times the ammonia added. This may be seen from the data at pH 5. Since the oxidation was not complete after 15 minutes, residuals after 60 minutes will be considered. The residual chlorine in the absence of ammonia was 24.2 p.p.m. and when 2 p.p.m. ammonia was added, 10.2 p.p.m., a drop in residual of 14.0 p.p.m., obviously a loss of 7 p.p.m. chlorine for each p.p.m. of ammonia that was added. Similar results were obtained at other reactions, the loss of chlorine ranging from 6 to 8 p.p.m. for each p.p.m. of ammonia employed.

When concentrations of 2 p.p.m. or less of ammonia were employed with 25 p.p.m. available chlorine (Series I, II and III), the initial chlorine, which consisted of hypochlorite was a good index to the probable germicidal efficiency. For example, at pH 7 (Series I, II and III) the initial chlorine concentrations were 23.0 p.p.m., 19.2 p.p.m. and 8.1 p.p.m. while the killing times were 3.0 minutes, 35 minutes and 6.5 minutes, respectively. Similar relationships were observed at more acid and more alkaline reactions (pH), although in the alkaline range, the killing times were distinctly longer.

Referring to Figures 7 and 7A it may be seen that with Series I, II and III, in which the residual chlorine existed as hypochlorite, the killing time became longer as the alkalinity (pH) increased. In general, the killing times when employing hypochlorite in the range pH 5 to pH 7 were not greatly different and were quite short, as may be seen in Series I (2.1 to 3.0 minutes) Series II (2.6 to 3.3 minutes) and Series III (4.4 to 6.5 minutes). At pH 8 the killing times were about $2\frac{1}{2}$ to 3 times those at pH 7. At pH 9 they were about 7 times those at pH 8, and at pH 10, the killing times were approximately 10 times those observed at pH 9, or 200 times those observed at pH 7. Thus, in the absence of ammonia (Series I) at pH 5 the killing time was 2.1 minutes and at pH 7, 3.0 minutes. When the reaction was slightly alkaline (pH 8) the killing time increased to 7.6 minutes and at more alkaline reactions, pH 9 and 10, the killing times

were 58 and 570 minutes respectively. The results of Series II and III were similar to those of Series I, but the killing times were longer because the initial hypochlorite concentrations were less.

Series IV and V, in which 6 and 18 p.p.m. ammonia, respectively, were added to 25 p.p.m. chlorine, resulting in the production of chloramines, show markedly different relationships of the effects of reaction (pH) and killing time. In the first place it will be noted that at pH 9 and more acid reactions, the chloramines were less efficient germicides than hypochlorites, but at the more alkaline reaction (pH 10), chloramines were more efficient than equal concentrations of hypochlorites. Referring to Figure 7 it may be seen that the curve for hypochlorite (Series I) crosses the curves for chloramines (Series IV and V) at about pH 9.3 and 9.6 respectively.

Another particularly interesting feature is a comparison of the influence of ammonia concentration (Series V) on the germicidal efficiency of chloramines. It will be noted in Table 7A that the residual chlorine in Series IV and V were remarkably constant. Such differences as were observed in the killing times for series IV and V at the various reactions (pH) would therefore be due to the effect of the ammonia present. The curve (Figures 7 and 7A) for Series V (18 p.p.m. of ammonia) crosses the curve for Series IV (6 p.p.m. of ammonia) at approximately pH 7.25. At the more acid reactions

the killing time was distinctly shorter with the higher ammonia concentration, whereas at the more alkaline reactions the reverse was the case.

The fact that at pH 5, the killing time for 22 to 23 p.p.m. available chlorine employing 6 p.p.m. ammonia was distinctly greater (168 minutes) than that obtained when 18 p.p.m. ammonia was added (99 minutes) throws some doubt on the view that dichloro-amine is more efficient as a germicide than monochloro-amine, since the higher ammonia concentration would tend toward the production of monochloro-amine.

TABLE 7

TIME IN MINUTES TO KILL 99% EXPOSED SPORES
(25 p.p.m. Av. Cl.; 20° C.)

Series	NH_3 (p.p.m.)	Av. Cl. / NH_3	pH 5.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 10.0
I	0.0		2.1	2.3	3.0	7.6	58	570
II	0.5	$\frac{50}{1}$	2.6	2.5	3.3	8.6	66	660
III	2	$\frac{12.5}{1}$	4.4	4.8	6.5	21	150	*
IV	6	$\frac{4.2}{1}$	168	85	89	83	182	186
V	8	$\frac{1.4}{1}$	99	59	84	107	263	456

* Killing time not reached in 12 hours at pH 10

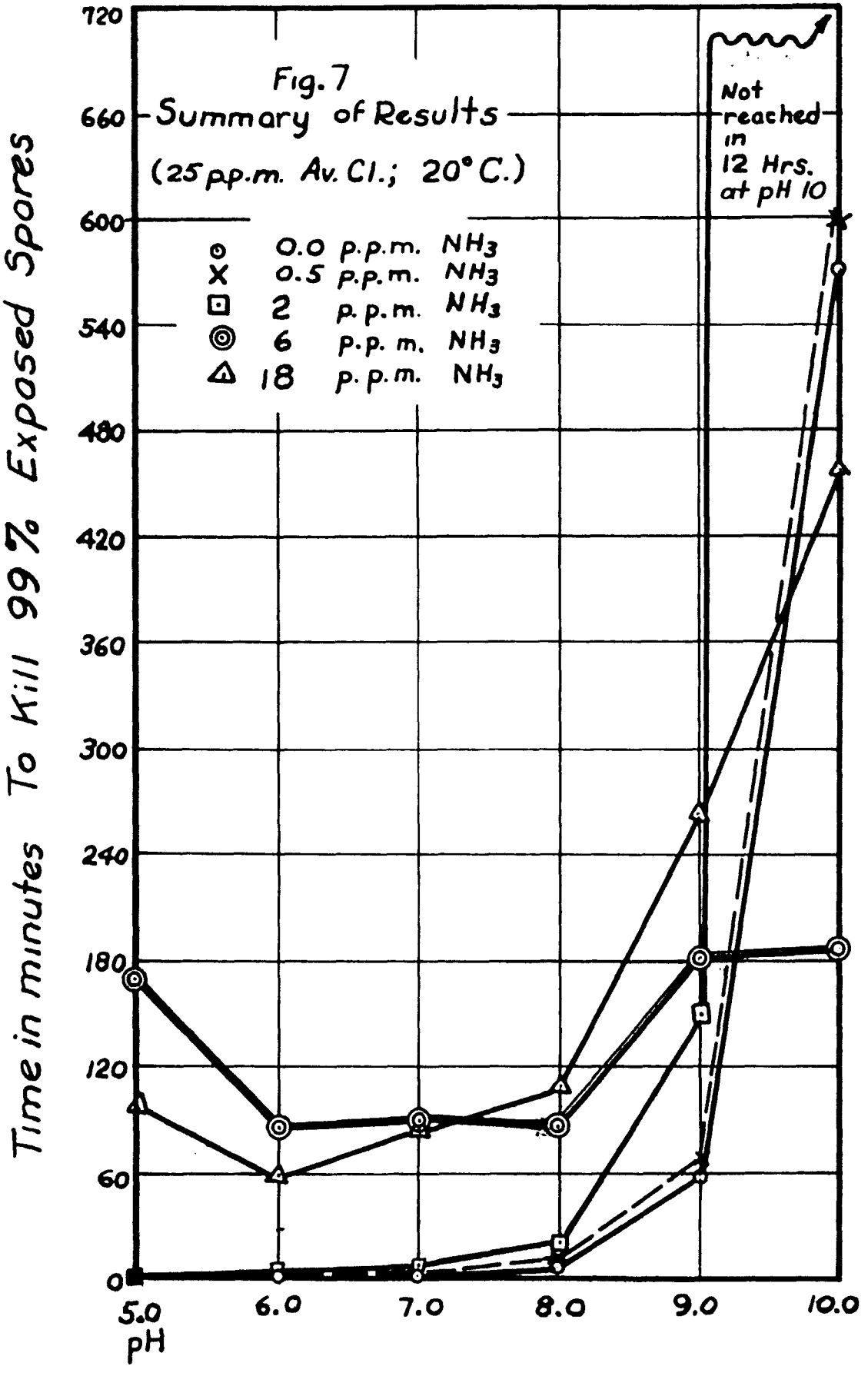


Fig. 7
Summary of Results
(25 p.p.m. Av. Cl.; 20° C.)
0.0 p.p.m. NH3
0.5 p.p.m. NH3
2 p.p.m. NH3
6 p.p.m. NH3
18 p.p.m. NH3
Not reached in 12 Hrs. at pH 10
Time in minutes To Kill 99% Exposed Spores
720
660
600
540
480
420
360
300
240
180
120
60
0
5.0
6.0
7.0
8.0
9.0
10.0
pH

TABLE 7A

EFFECT OF REACTION (pH) AND AMMONIA ON RESISTENCE OF CHLORINE
(25 p.p.m. Av. Cl. added; 20° C.)

Series	NH_3 p.p.m. Added	Ratio Av. Cl. / NH_3 Added	pH 5.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 10.0
Residual Av. Cl. (initial) after 15 minutes contact at 20° C.#*								
I	0.0		24.2	23.4	23.0	22.0	24.2	24.2
II	0.5	50/1	21.1	19.5	19.2	18.6	20.3	21.1
III	2	12.5/1	14.0	10.4	8.1	7.5	11.1	13.3
IV	6	4.2/1	22.6	21.8	23.6	22.0	23.0	23.0
V	18	1.4/1	23.3	23.1	23.4	22.8	22.6	23.0
Residual Av. Cl. after 60 minutes contact at 20° C. #*								
IA	0.0		24.2	23.4	23.2	22.0	24.2	24.2
IIA	0.5	50/1	21.3	19.8	19.4	18.6	20.4	21.1
IIIA	2	12.5/1	10.2	10.8	7.3	7.7	10.9	11.7
IVA	6	4.2/1	22.1	21.8	21.1	19.6	22.6	23.2
VA	18	1.4/1	23.4	23.4	23.0	21.6	22.9	23.1
Residual Av. Cl. at end of experiment (20° C.)								
IB	0.0		24.2 *	22.3 *	22.0 *	22.9 *	21.7 ***	20.3 *#
IIB	0.5	50/1	21.8 *	19.7 *	19.0 *	18.9 *	17.8 ***	16.8 *#
IIIB	2	12.5/1	11.4 *	10.2 *	8.7 *	8.2 **	7.8 ##	8.1 *#
IVB	6	4.2/1	22.0 ##	20.2 #	21.1 #	22.3 #	17.9 ##	20.6 ##
VB	18	1.4/1	23.4 #	22.4 ***	23.8 #	22.9 ##	20.2 ###	21.0 *#

* Less than 30 minutes contact
** 30 to 60 minutes contact
*** 75 to 100 minutes contact
\# 100 to 150 minutes contact
150 to 250 minutes contact
250 to 350 minutes contact
*# 450 to 740 minutes contact
#* Residual Av. Cl. after ___ minutes contact of chlorine with buffered solution

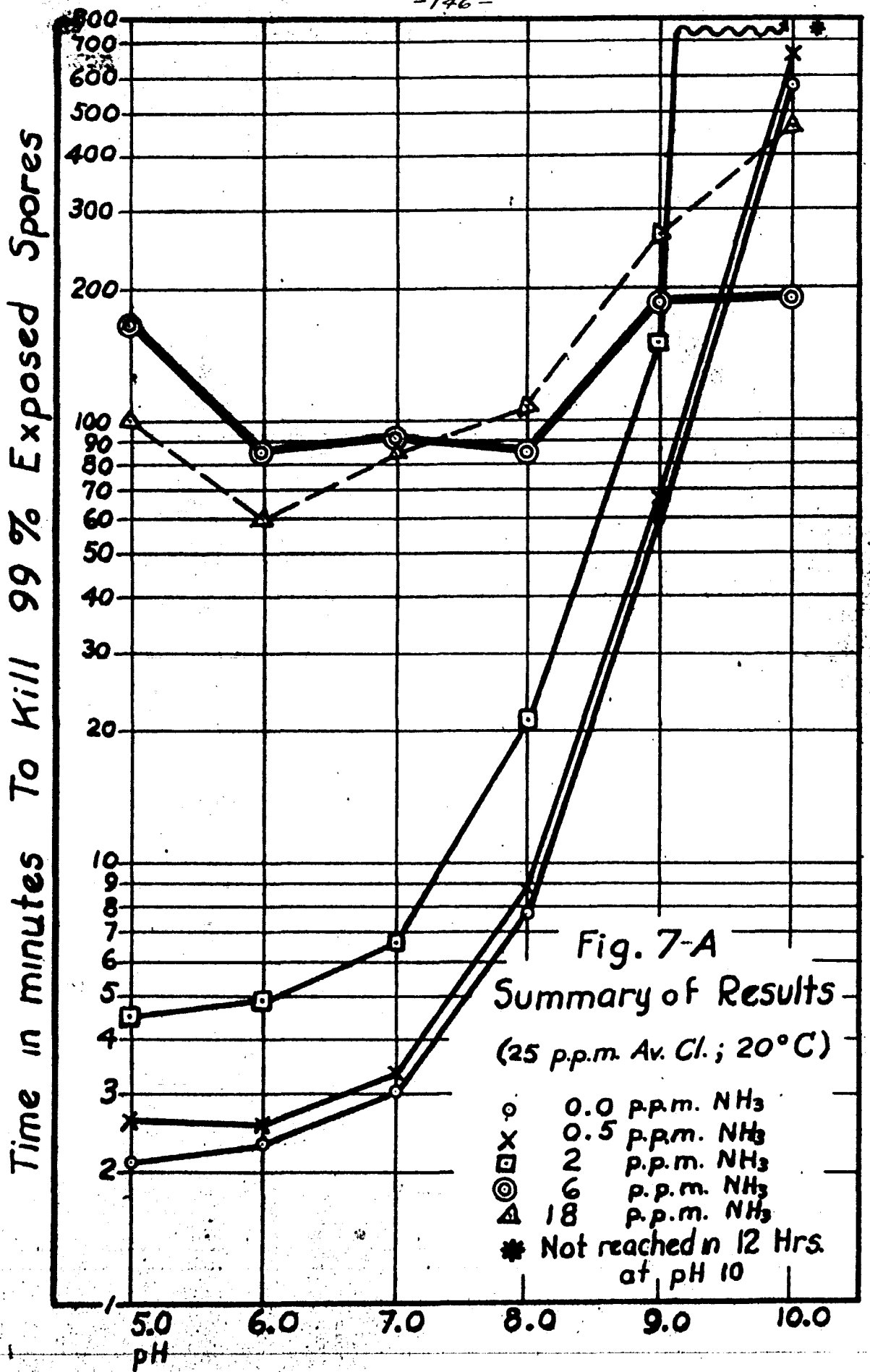


Fig. 7-A

Summary of Results

(25 p.p.m. Av. Cl.; 20° C)

2. Effect of concentration on the germicidal efficiency of chlorine at 20° C. and pH 10.

In order to ascertain the effect of concentration on the germicidal efficiency of chlorine, 25, 50, 100 and 200 p.p.m. available chlorine were added to buffered water at pH 10 (20° C.) and after 15 minutes, B. metiens spores were subjected to the action of these solutions. Data for these experiments are presented in Table 8 and survivor curves are shown graphically in Figure 8. Table 8A and Figure 8A show the relation of chlorine concentration to the resistance of B. metiens spores. In Table 8B are shown the residual chlorine concentration 15 minutes after the solutions were compounded. Since the B. metiens spores were added at this time, this residual is the initial chlorine concentration to which the test organism was exposed.

The solution to which 25 p.p.m. available chlorine were added showed a concentration of 24.2 p.p.m. available chlorine at the time the experiment was begun (15 minutes after the addition of the chlorine) and a residual of 20.3 p.p.m. at the end of the experiment. It should be pointed out however that this residual, determined at the end of the experiment, was made after a rather long exposure time since the killing time was 570 minutes. The solutions to which 50, 100 and 200 p.p.m. available chlorine were added showed initial chlorine concentrations of 46.5, 92.3 and

188 p.p.m., respectively, with no appreciable change during the course of the experiments. The respective killing times for these three concentrations of chlorine were 291, 170 and 98 minutes. It will be noted that the reaction was constant, varying only between pH 9.98 and 10.02.

Figure 8A shows that when the logarithm of the initial concentration of available chlorine is plotted against the logarithm of the killing time the points fall on a straight line. The equation for this line is:

$$\log y = (-0.860) \log x + 3.936$$

where y is the killing time in minutes and x is the initial concentration of available chlorine in p.p.m.

In general, it may be said that when the concentration of available chlorine was doubled, the killing time was reduced by about 60 percent.

TABLE 8

EFFECT OF CHLORINE CONCENTRATION ON RESISTANCE OF B. METIENS SPORES
(20° C.; pH 10.0)

Av. Cl. p.p.m. added	25			50				
Expt. No.	*			Expt. No.	176	182		
Date		Av. % Survivors*	Log. Av. % Survivors*	Date	4/3/40	4/8/40	Av. % Survivors	Log. Av. % Survivors
Exposure Time (in min.)				Exposure Time (in min.)	** Survivors	** Survivors		
0		100	2.00	0	850	1,000	100	2.00
60		95	1.98	30		1,150		
120		120	2.08	60	850	900	95	1.98
180		94	1.97	90		1,150		
240		78	1.89	120	800	1,000	97	1.99
300		60	1.78	150		800		
360		29	1.46	180	500	300	45	1.65
420		6.8	0.83	210		95		
480		3.3	0.52	240	28.5	34.5	3.4	0.53
540		1.9	0.28	270		19.5		
600		0.74	1.87	300	6.5	5.5	0.66	1.82
660								

Av. Cl. p.p.m. added	100				200				
Expt. No.	174	180			Expt. No.	172	178		
Date	4/2/40	4/7/40	% Survivors#	Log % Survivors	Date	4/2/40	4/7/40	% Survivors##	Log % Survivors
Exposure Time (in min.)	** Survivors	** Survivors			Exposure Time (in min.)	** Survivors	** Survivors		
0	1,000	950	100	2.00	0	950	900	100	2.00
20		950	100	2.00	15		950	105	2.02
40		1,100	115	2.06	30	800	950	105	2.02
60	750	850	90	1.95	45		800	89	1.95
80		700	74	1.87	60	65	100	11	1.04
100		475	50	1.70	75		48	5.3	0.73

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540		1.9	0.28	270		19.5		
600		0.74	1.87	300	6.5	5.5	0.66	1.82
660								

Av. Cl. p.p.m. added	100				200				
Expt. No.	174	180			Expt. No.	172	178		
Date	4/2/40	4/7/40	%	Log	Date	4/2/40	4/7/40	%	Log
Exposure Time (in min.)	Survivors **	Survivors **	Survivors#	% Survivors	Exposure Time (in min.)	Survivors **	Survivors **	Survivors##	% Survivors
0	1,000	950	100	2.00	0	950	900	100	2.00
20		950	100	2.00	15		950	105	2.02
40		1,100	115	2.06	30	800	950	105	2.02
60	750	850	90	1.95	45		800	89	1.95
80		700	74	1.87	60	65	100	11	1.04
100		475	50	1.70	75		48	5.3	0.73
120	40.5	115	12	1.08	90	3.3	18.5	2.1	0.31
140		33	3.5	0.54	105		0.9	0.10	1.00
160		18	1.9	0.28					
180		5	0.53	1.72					

Av. Cl. Added (p.p.m.)	25		50			100			200		
Expt. No.	*	Average	176	182	Average	174	180	Average	172	178	Average
Av. Cl. Residual at end of experiment		20.3	46.0	48.8	47.4	97.7	93.1	95.4	185	189	187
pH at end of experiment		9.98	9.98	10.00	9.99	9.90	10.02	9.96	9.95	10.02	9.99
Killing time (min.) ***		570	294	288	291	###	170	----	###	98	----

* See Table 6
** Surviving bacteria (in thousands) per 5 ml.
*** Time for killing 99%
\# For Expt. No. 180 only
For Expt. No. 178 only
Not determined

Fig. 8

EFFECT OF CHLORINE CONCENTRATION ON RESISTANCE OF B. METIENS SPORES
(20° C.; pH 10.0; Chlorine Expressed as p.p.m. Av. Cl. Added)

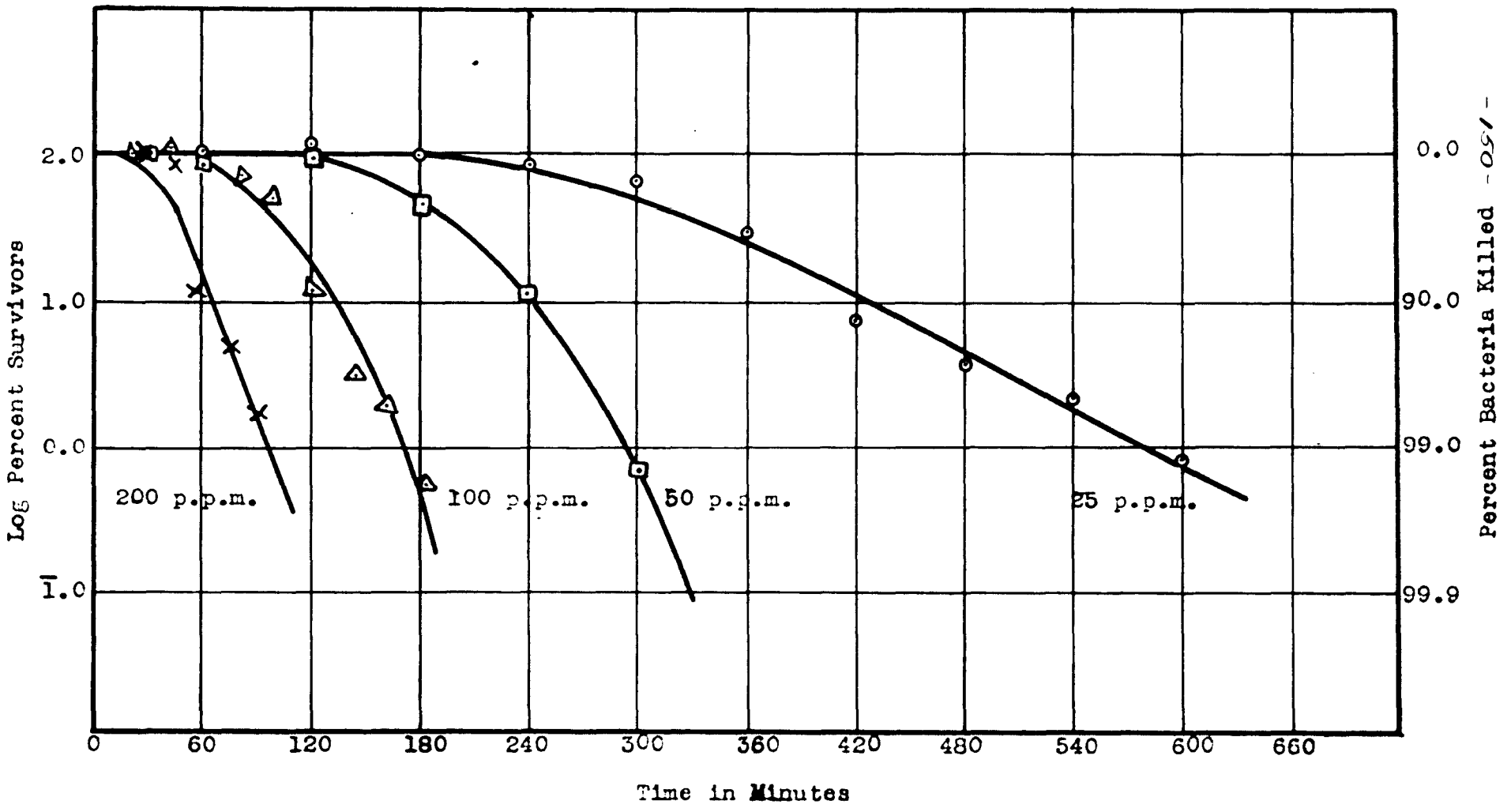


TABLE 8A

RELATION OF CHLORINE CONCENTRATION TO RESISTANCE OF B. MYCOIDES SPORES
(20° C.; pH 10.0)

Av. Cl. p.p.m. (added)	Residual Av. Cl. after 15 min. (initial)	% of Av. Cl. added remaining after 15 min.	Time (min.) to kill 99 %	Log time (min.) to kill 99 %	Log Residual Av. Cl. after 15 min.	Ratio times (min.) to kill 99 %
25#	24.2	97	570	2.76	1.38	----
50	46.5	93	291	2.46	1.67	$\frac{570}{291} = 2.0$
100	92.3	92	170	2.23	1.97	$\frac{291}{170} = 1.7$
200	188	94	98	1.99	2.27	$\frac{170}{98} = 1.7$

See Table 6 and Fig. 6.

Fig. 8A

RELATION OF CHLORINE CONCENTRATION TO RESISTANCE OF B. METIENS SPORES

(20° C.; pH 10.0)

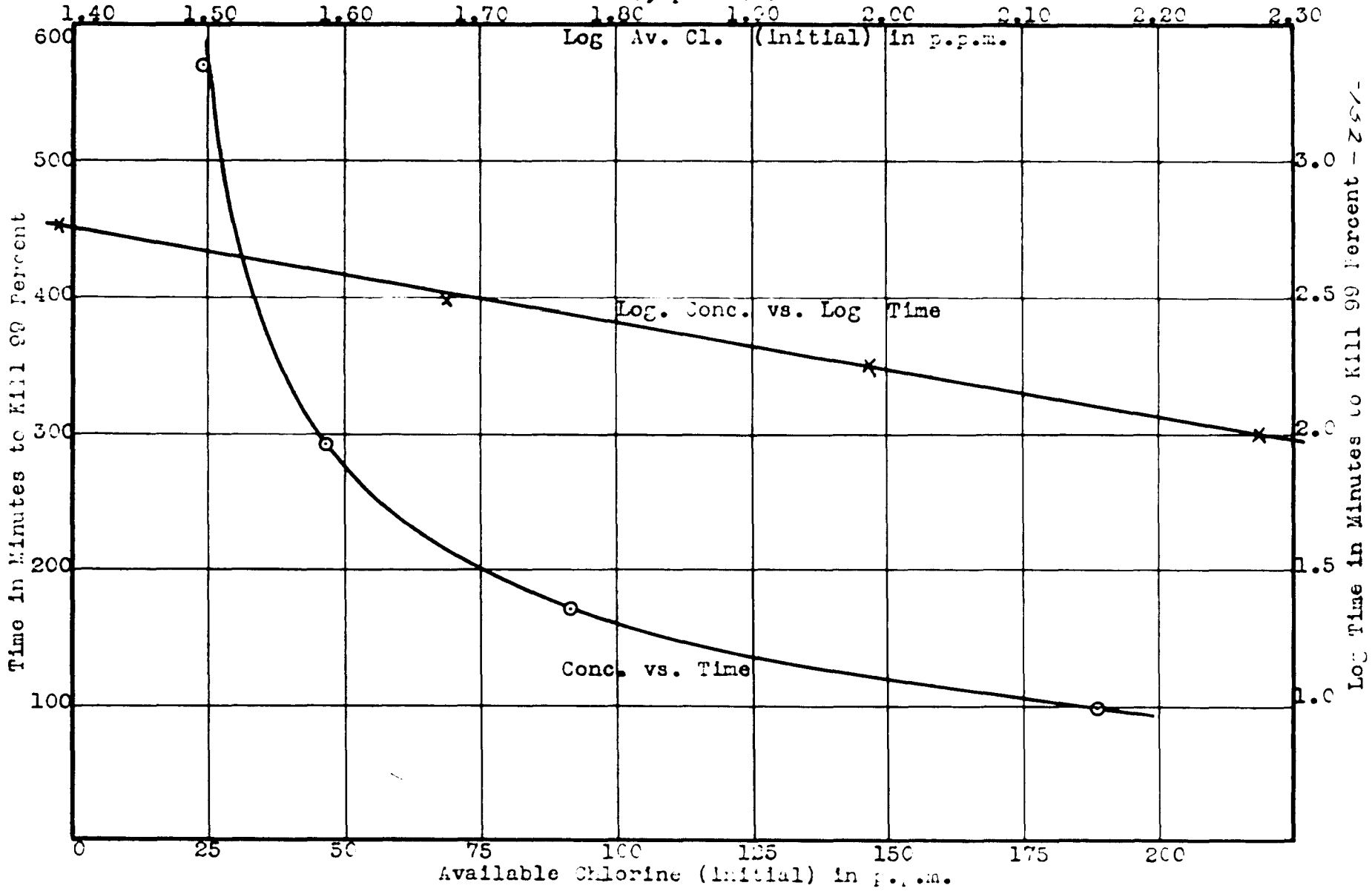


TABLE 8B

SUMMARY OF DATA SHOWING EFFECT OF CHLORINE CONCENTRATION ON RESISTANCE OF B. MYCOIDES SPORES

(20° C; pH 10.0; see Tables 8 and 8A)

Concentration (p.p.m.) Av. Cl. added	25	50	100	200
Residual Av. Cl. (p.p.m.) after 15 min. contact#	24.2	46.5	92.3	187
Residual Av. Cl. (p.p.m.) at end of experiment	20.3	47.4	93.1	189
Killing time* (min.)	570	291	170	98
pH at end of experiment	9.98	9.99	10.02	10.02

* Time in minutes to kill 99% exposed spores.

Residual Av. Cl. after 15 minutes contact of chlorine with the buffered solution.

3. Effect of concentration of the germicidal efficiency of chlorine-ammonia solutions at 20° C. and pH 10.

The following experiments were carried out in solutions buffered at pH 10 (20° C.) containing 6, 12, 24 and 48 p.p.m. ammonia to which were added 25, 50, 100 and 200 p.p.m. available chlorine, respectively, so as to produce monochloro-amine. Results for these experiments are presented in Table 9 and survivor curves are shown graphically in Figure 9. The relation of chlorine-ammonia concentration to killing time is shown in Table 9A and Figure 9A. Residual chlorine concentrations at the time that the bacteria were introduced (15 minutes after the addition of chlorine) and at the end of the experiment are given in Table 9B. The reaction (pH) was constant throughout all of the experiments, varying only from pH 9.99 to 10.02.

When 25 p.p.m. available chlorine and 6 p.p.m. ammonia were added to the buffered solution at 20° C. and pH 10, the initial chlorine 15 minutes after the solutions were compounded (the time at which the test spores were added) was 23.0 p.p.m. The residual determined at the end of the experiment showed that 20.6 p.p.m. available chlorine were present and the killing time was 186 minutes.

When the concentrations of both chlorine and ammonia were doubled (50 p.p.m. chlorine and 12 p.p.m. ammonia) the

initial chlorine concentration was 41.5 p.p.m., the residual at the end of the experiment was 40.8 p.p.m. and the killing time was 95 minutes.

When employing 100 p.p.m. available chlorine with 24 p.p.m. ammonia the initial chlorine concentration was 84.6 p.p.m. and the residual had dropped to 80.0 p.p.m. by the time the experiment was completed. The time required to kill 99 percent of the exposed spores was 60 minutes.

When the concentrations were again doubled (200 p.p.m. available chlorine and 48 p.p.m. ammonia added) the initial chlorine was 140 p.p.m. with no appreciable change during the experiment. The killing time was 51 minutes.

It will be noted in Figure 9A that plotting the logarithm of the initial chlorine concentration in p.p.m. against the logarithm of the killing time in minutes gives a slightly curved line. A killing time of 186 minutes employing 23.0 p.p.m. available chlorine as chloramine dropped to 95 minutes (a reduction of 49 percent) when the concentration of chloramine was approximately doubled (41.5 p.p.m. available chlorine). Again doubling the chloramine (to 84.6 p.p.m.) resulted in a decrease in killing time by only 37 percent, to 60 minutes. With 140 p.p.m. available chlorine as chloramine, the killing time was 51 minutes, or a reduction of only 15 percent from that obtained with about 85 p.p.m. available chlorine. It appears, therefore, that as the concentration of chloramine increased, the relative decrease in killing time progressively decreased.

TABLE 9

EFFECT OF CHLORINE-AMMONIA CONCENTRATION ON RESISTANCE OF B. METIENS SPORES

(Ratio of $\frac{\text{Av. Cl.}}{NH_3}$ = $\frac{25}{6}$ in p.p.m. added; 20° C.; pH 10.0)

Av. Cl. p.p.m. added	25			50				
NH_3 p.p.m. added	6			12				
Expt. No.	*			Expt. No.	175	181		
Date				Date	4/31/40	4/8/40		
Exposure Time (in min.)		Av. % Survivors*	Log. Av. % Survivors*	Exposure Time (in min.)	** Survivors	** Survivors	% Survivors#	Log % Survivors
0		100	2.00	0	1,100	850	100	2.00
30		53	1.72	20	430	650	76	1.88
60		23	1.36	40		120	14	1.15
90		12	1.08	60	18.5	46.5	5.5	0.74
120		5.4	0.73	80		18	2.1	0.33
150		1.9	0.29	100		8	0.94	1.97
180		1.0	0.00	120	3	1.8	0.21	1.33
210		0.48	1.68					
240		----	----					
270		----	----					

Av. Cl. p.p.m. added	100				200			
NH_3 p.p.m. added	24				48			
Expt. No.	173	179			Expt. No.	171	177		
Date	4/2/40	4/7/40			Date	4/2/40	4/7/40		
Exposure Time (in min.)	** Survivors	** Survivors	% Survivors##	Log % Survivors	Exposure Time (in min.)	** Survivors	** Survivors	% Survivors###	Log % Survivors

270

Av. Cl. p.p.m. added	100				200				
NH_3 p.p.m. added	24				48				
Expt. No.	173	179			Expt. No.	171	177		
Date	4/2/40	4/7/40	%	Log	Date	4/2/40	4/7/40	%	Log
Exposure Time (in min.)	** Survivors	** Survivors	Survivors##	% Survivors	Exposure Time (in min.)	** Survivors	** Survivors	Survivors###	% Survivors
0	1,050	800	100	2.00	0	650	700	100	2.00
10	225	500	63	1.80	10	550	475	68	1.83
20	190	270	34	1.53	20	165	110	16	1.20
30	65	135	17	1.23	30	80	50	7.1	0.85
40	----	29.5	3.7	0.57	40	---	26	3.7	0.57
50	----	15	1.9	0.27	50	---	11	1.6	0.20
60	3	9	1.1	0.05	60	3.25	2.8	0.40	$\bar{1}$.60
70	----	4.25	0.53	$\bar{1}$.73	70	---	2	0.29	$\bar{1}$.46

Av. Cl. Added (p.p.m.)	25		50			100			200		
NH_3 Added (p.p.m.)	6		12			24			48		
Expt. No.	*	Average	175	181	Average	173	179	Average	171	177	Average
Av. Cl. Residual at end of experiment		20.6	42.0	40.8	41.4	81.4	80.0	80.7	139	138	139
pH at end of experiment		9.99	9.98	10.00	9.99	9.95	10.02	9.99	9.95	10.02	9.99
Killing time (min.) ***		186	*#	95	----	*#	60	----	54	51	*#

* See Table 6C
** Surviving bacteria (in thousands) per 5 ml.
*** Time for killing 99%
\# For Expt. No. 181 only
For Expt. No. 179 only
For Expt. No. 177 only
*# Not determined

Fig. 9

EFFECT OF CHLORINE-AMMONIA CONCENTRATION ON RESISTANCE OF B. METIENS SPORES

(Ratio of $\frac{\text{Av. Cl.}}{NH_3} = \frac{25}{6}$ in p.p.m. added; 20° C.; pH 10.0)

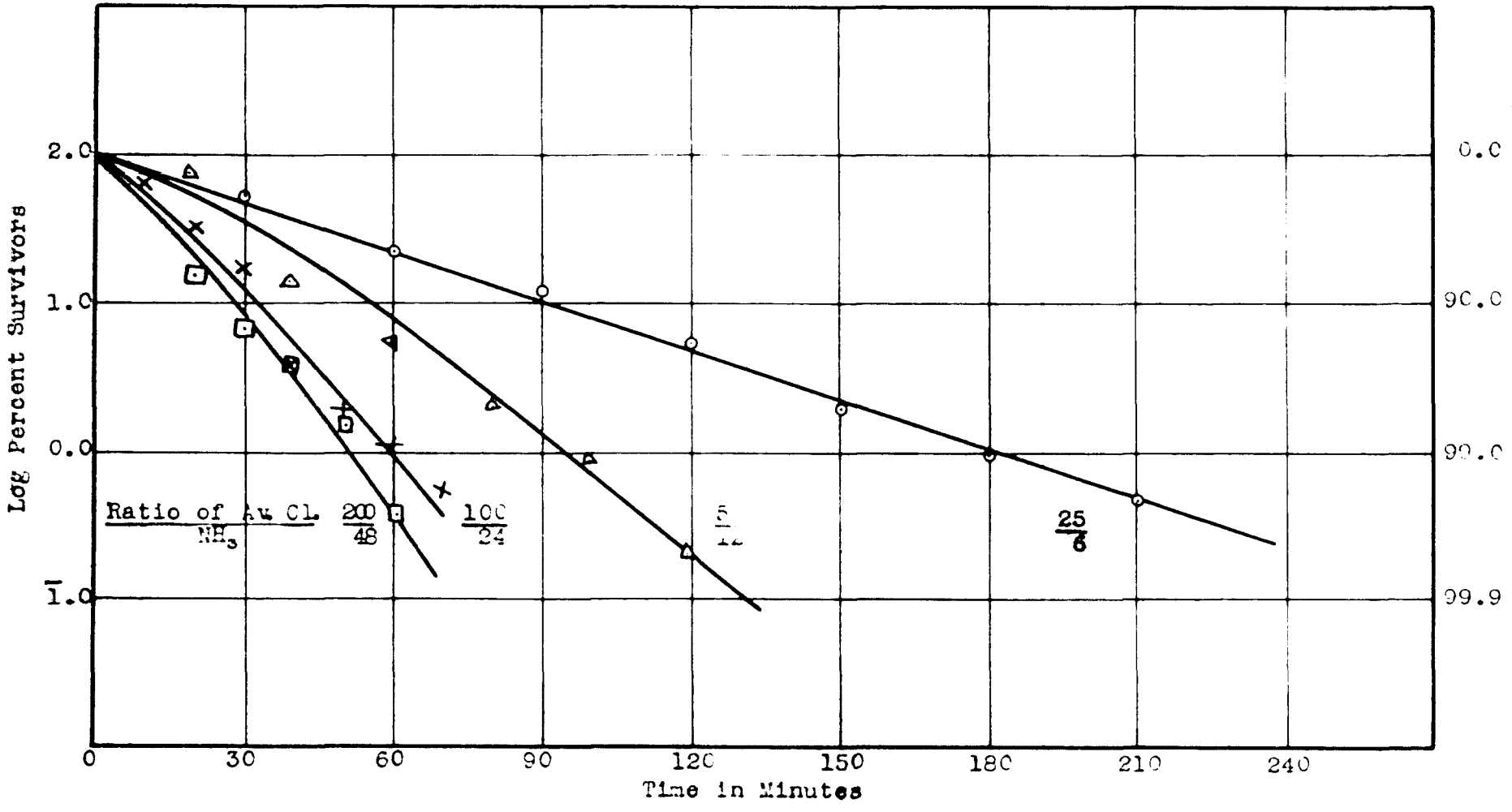


TABLE 9A

RELATION OF INITIAL CHLORINE CONCENTRATION TO RESISTANCE OF B. METIENS SPORES IN CHLORINE-AMMONIA SOLUTION

(Ratio of $\frac{\text{Av. Cl.}}{NH_3}$ = $\frac{25}{6}$ in p.p.m. added; 20° C.; pH 10.0)

Av. Cl. p.p.m. (added)	NH_3 p.p.m. (added)	Residual Av. Cl. after 15 min. (initial	% of Av. Cl. added remaining after 15 min.	Time (min.) to kill 99%	Log Time (min.) to kill 99%	Log residual Av. Cl. after 15 min.	Ratio Times (min.) to kill 99%
25#	6	23.0	92	136	2.27	1.36	-----
50	12	41.5	83	95	1.98	1.62	$\frac{186}{95}$ = 2.0
100	24	84.6	85	60	1.78	1.93	$\frac{95}{60}$ = 1.6
200	48	140	70	51	1.71	2.15	$\frac{60}{51}$ = 1.2

See Table 6C and Fig. 6C.

FIG. 9A

RELATION OF INITIAL CHLORINE CONCENTRATION AND TIME REQUIRED TO KILL 99 PERCENT OF B. TYPHOSUS IN SURFACE-WATER SOLUTION

(Slope of $\frac{\text{Log. Available Chlorine (Initial)}}{\text{Log. Time}} = \frac{-c}{t}$; $c = 0.5$; $t = 1.0$)

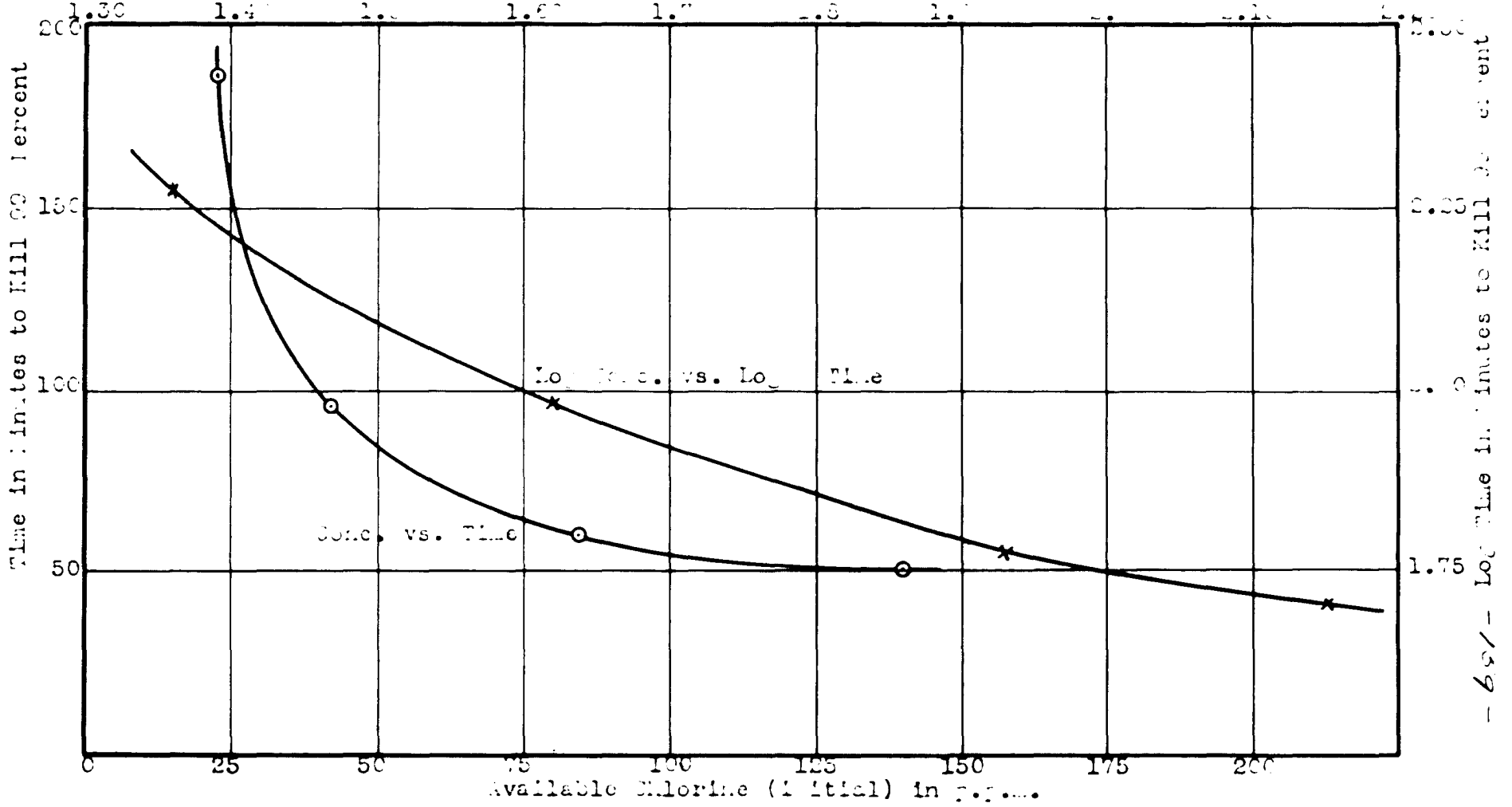


TABLE 9B

SUMMARY OF DATA SHOWING EFFECT OF CHLORINE-AMMONIA CONCENTRATION ON RESISTANCE OF B. METIENS SPORES

(Ratio $\frac{\text{Av. Cl.}}{NH_3} = \frac{25}{6}$ in p.p.m. added; 20° C.; pH 10.0; See Tables 9 and 9A)

Concentration (p.p.m.) Av. Cl. added	25	50	100	200
Concentration of NH_3 added (p.p.m.)	6	12	24	48
Residual Av. Cl. (p.p.m.) after 15 min. contact #	23.0	41.5	84.6	140
Residual Av. Cl. (p.p.m.) at end of experiment	20.6	40.8	80.0	138
Killing time* (min.)	186	95	60	51
pH at end of experiment	9.99	10.00	10.02	10.02

* Time in minutes to kill 99% exposed spores.

Residual Av. Cl. after 15 minutes contact of chlorine with the buffered solution.

In Table 9C the killing times for the various concentrations of chlorine and chlorine-ammonia (chloramine) solutions (at pH 10) are presented. These data are shown graphically in Figure 9B. With all concentrations employed, shorter killing times were obtained with chlorine-ammonia mixtures than with chlorine alone. In the upper right-hand quadrant of Figure 9B are the graphs comparing chlorine and chlorine-ammonia solutions, when the logarithm of the initial chlorine concentration in p.p.m. is plotted against the logarithm of the killing time in minutes. It will be observed that the line for chlorine solution is straight, while that for chlorine-ammonia solution is slightly curved. If these two lines were extrapolated they would intercept. It seems that some concentration of available chlorine either as hypochlorite or chloramine would be equally effective as a germicide. From the graph it appears that this concentration would be approximately 400 p.p.m. available chlorine.

TABLE 9C

SHOWING INFLUENCE OF CONCENTRATION ON THE RESISTANCE OF B. METIENS SPORES TO CHLORINE AND CHLORINE-AMMONIA SOLUTIONS

(20° C.; pH 10.0; See Tables 8A and 9A)

Av. Cl. (p.p.m.)#	Chlorine		Chlorine-Ammonia	
	NH_3 (p.p.m)	Killing Time*	NH_3 (p.p.m.)	Killing Time*
25	0	570	6	186
50	0	291	12	95
100	0	170	24	60
200	0	98	48	51

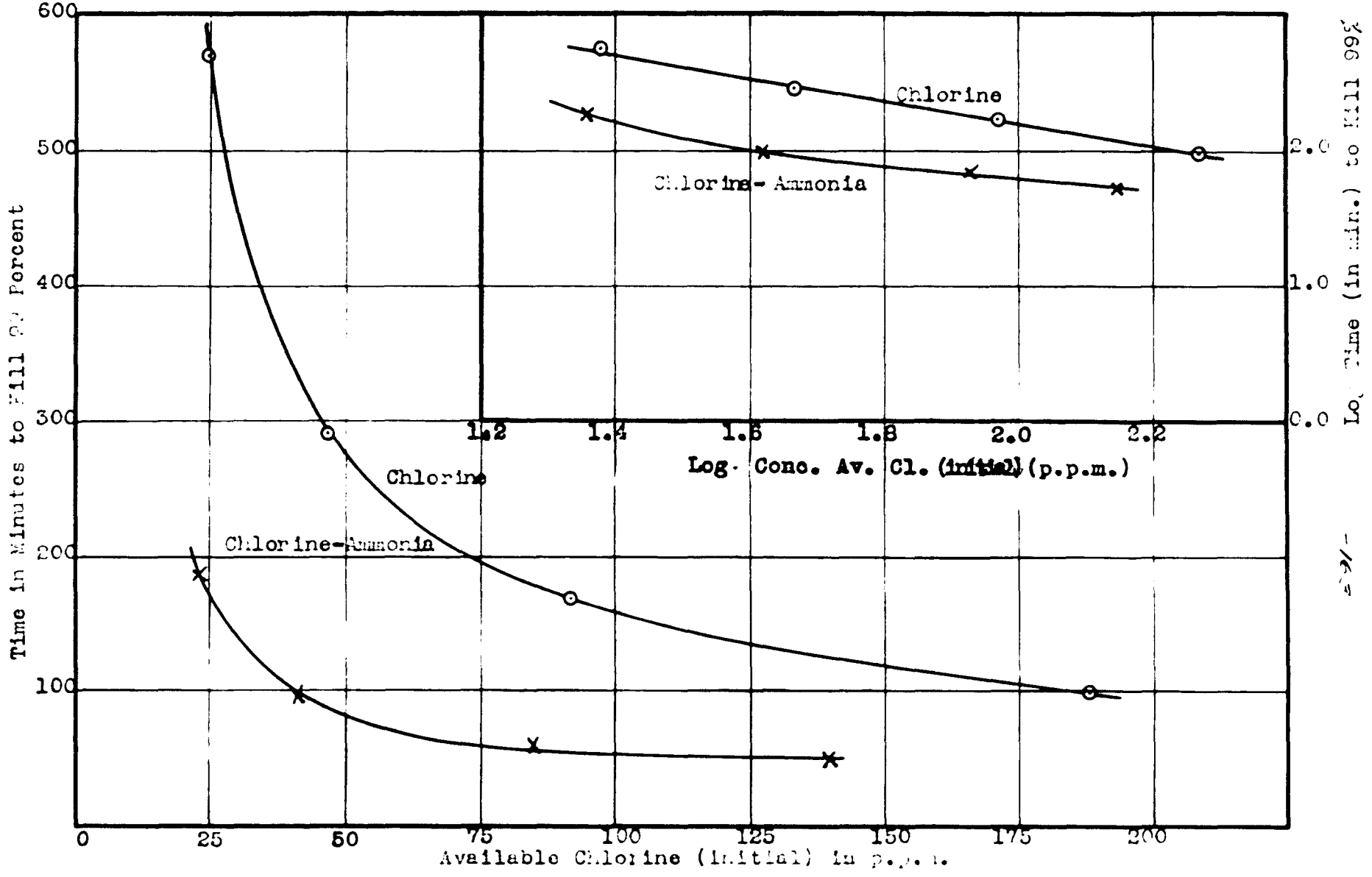
* Time to kill 99% exposed spores

Available chlorine added

Fig. 9B

SHOWING INFLUENCE ON CONCENTRATION ON THE RESISTANCE OF B. METIENS SPORES TO CHLORINE AND CHLORINE-AMMONIA SOLUTION

(20° C.; pH 10.0; see Fig. 8A and Fig. 9A)



4. Effect of temperature on the germicidal efficiency of chlorine solutions at pH 10.

Twenty-five p.p.m. of chlorine were added to a buffered solution (pH 10) at temperatures of 20° C., 30° C., 40° C., and 50° C. The data are detailed in Table 10 and the results are shown graphically in Figure 10. Table 10A shows the influence of temperature on the germicidal efficiency of chlorine and these data are presented graphically in Figure 10A. Table 10B shows the initial chlorine concentrations after 15 minutes, (the time at w ich the test organism was added) and the residual chlorine concentrations at 20° C., 30° C., 40° C. and 50° C. determined at the end of the experiments. It will be noted that the initial chlorine was not significantly different at the various temperatures.

At 20° C., the initial chlorine concentration was 24.2 p.p.m. and this dropped to 20.3 p.p.m. at the end of the experiment. It required 570 minutes to kill 99 percent of the exposed spores.

At 30° C. the initial concentration of chlorine was 23.3 p.p.m., the residual at the end of the experiment was 20.5 p.p.m., and the killing time was 240 minutes.

When the temperature was raised to 40° C., 23.2 p.p.m. chlorine was the initial concentration at the time the test

organism was added, and this dropped to 19.8 p.p.m. at the end of the experiment. The killing time was 100 minutes.

Increasing the temperature to 50° C. resulted in an initial chlorine concentration of 22.8 p.p.m. During the experiment the residual dropped to 19.3 p.p.m. and the killing time was 46 minutes.

The reaction (pH) was constant (9.98) throughout all of the experiments.

In Figure 10A it will be noted that when the logarithms of the killing times were plotted against the logarithms of the corresponding temperatures, the resulting graph closely approximated a straight line, the equation for which is

$$\log y = (-0.370) \log x + 2.334$$

where y is temperature in degrees centigrade and x is the killing time in minutes.

By increasing the temperature 10° C., the killing time was shortened by about 60 percent.

TABLE 10

EFFECT OF TEMPERATURE ON RESISTANCE OF B. METIENS SPORES TO CHLORINE SOLUTION
(25 p.p.m. Av. Cl. added; pH 10.0)

Temperature Degrees C.	20			30				
Expt. No.	*			Expt. No.	165	167		
Date				Date	3/22/40			
Exposure Time (in min.)		Av. % Survivors*	Log Av. % Survivors*	Exposure Time (in min.)	** Survivors	** Survivors	% Survivors#	Log % Survivors
0		100	2.00	0	1,200	1,600	100	2.00
60		95	1.98	30	-----	1,750	110	2.04
120		120	2.08	60	1,250	1,550	97	1.99
180		94	1.97	90	-----	1,400	88	1.94
240		78	1.89	120	1,250	1,150	72	1.86
300		60	1.78	150	-----	900	56	1.75
360		29	1.46	180	40.5	190	12	1.08
420		6.8	0.83	210	-----	38	2.4	0.38
480		3.3	0.52	240	15	13	0.81	I.91
540		1.9	0.28	270	-----	6.5	0.41	I.61
600		0.74	I.87	300	1.5			
660		---	----					

Temperature Degrees C.	40				50				
Expt. No.	163	170			Expt. No.	158	161		
Date	3/21/40	3/28/40			Date	3/9/40	3/21/40		
Exposure Time (in min.)	** Survivors	** Survivors	Av. % Survivors	Log Av. % Survivors	Exposure Time (in min.)	** Survivors	** Survivors	% Survivors##	Log % Survivors
0	1,700	950	100	2.00	0	900	1,650	100	2.00
30	-----	-----			30	-----	1,600	97	1.99
60	1,600	1,000	88	1.94	60	1,000	1,450	88	1.94
90	-----	-----			90	-----	1,250	76	1.88
120	1,450	1,000	82	1.91	120	1,000	1,150	70	1.84
150	-----	-----			150	-----	1,000	61	1.78
180	1,250	1,000	74	1.87	180	1,000	1,000	61	1.78
210	-----	-----			210	-----	1,000	61	1.78
240	1,250	1,000	74	1.87	240	1,000	1,000	61	1.78
270	-----	-----			270	-----	1,000	61	1.78
300	1,250	1,000	74	1.87	300	1,000	1,000	61	1.78
330	-----	-----			330	-----	1,000	61	1.78
360	1,250	1,000	74	1.87	360	1,000	1,000	61	1.78
390	-----	-----			390	-----	1,000	61	1.78
420	1,250	1,000	74	1.87	420	1,000	1,000	61	1.78
450	-----	-----			450	-----	1,000	61	1.78
480	1,250	1,000	74	1.87	480	1,000	1,000	61	1.78
510	-----	-----			510	-----	1,000	61	1.78
540	1,250	1,000	74	1.87	540	1,000	1,000	61	1.78
570	-----	-----			570	-----	1,000	61	1.78
600	1,250	1,000	74	1.87	600	1,000	1,000	61	1.78
630	-----	-----			630	-----	1,000	61	1.78
660	1,250	1,000	74	1.87	660	1,000	1,000	61	1.78

360		29	1.46	180	40.5	190	12	1.08
420		6.8	0.83	210	-----	38	2.4	0.38
480		3.3	0.52	240	15	13	0.81	I.91
540		1.9	0.28	270	-----	6.5	0.41	I.61
600		0.74	I.87	300	1.5			
660		---	----					

Temperature Degrees C.	40				50				
Expt. No.	163	170			Expt. No.	158	161		
Date	3/21/40	3/28/40	Av. % Survivors	Log Av. % Survivors	Date	3/9/40	3/21/40	% Survivors##	Log % Survivors
Exposure Time (in min.)	** Survivors	** Survivors			Exposure Time (in min.)	** Survivors	** Survivors		
0	1,700	950	100	2.00	0	900	1,650	100	2.00
20	1,150	900	82	1.91	10	900	2,000	120	2.08
40	1,350	800	84	1.92	20	900	1,400	85	1.93
60	950	550	57	1.76	30	---	650	40	1.60
80	110	210	14	1.15	40	70	80	4.9	0.69
100	14	10	0.96	I.98	50	---	6.5	0.35	I.55
120	2	2	0.16	I.20					

Temperature Degrees C.	20		30			40			50		
Expt. No.	*	Average	165	167	Average	163	170	Average	158	161	Average
Av. Cl. Residual at end of experiment		20.3	19.0	20.5	19.8	19.6	20.0	19.8	19.7	19.3	19.5
pH at end of experiment		9.98	9.98	9.98	9.98	9.98	9.97	9.98	10.00	9.98	9.99
Killing time (min.) ***		570	246	240	###	98	101	100	###	46	-----

* See Table 6
** Surviving bacteria (in thousands) per 5 ml.
*** Time for killing 99%
\# For Expt. No. 167 only
For Expt. No. 161 only
Not determined

Fig. 10

EFFECT OF TEMPERATURE ON RESISTANCE OF B. METIENS SPORES TO CHLORINE SOLUTION
(25 p.p.m. Av. Cl. added; pH 10.0)

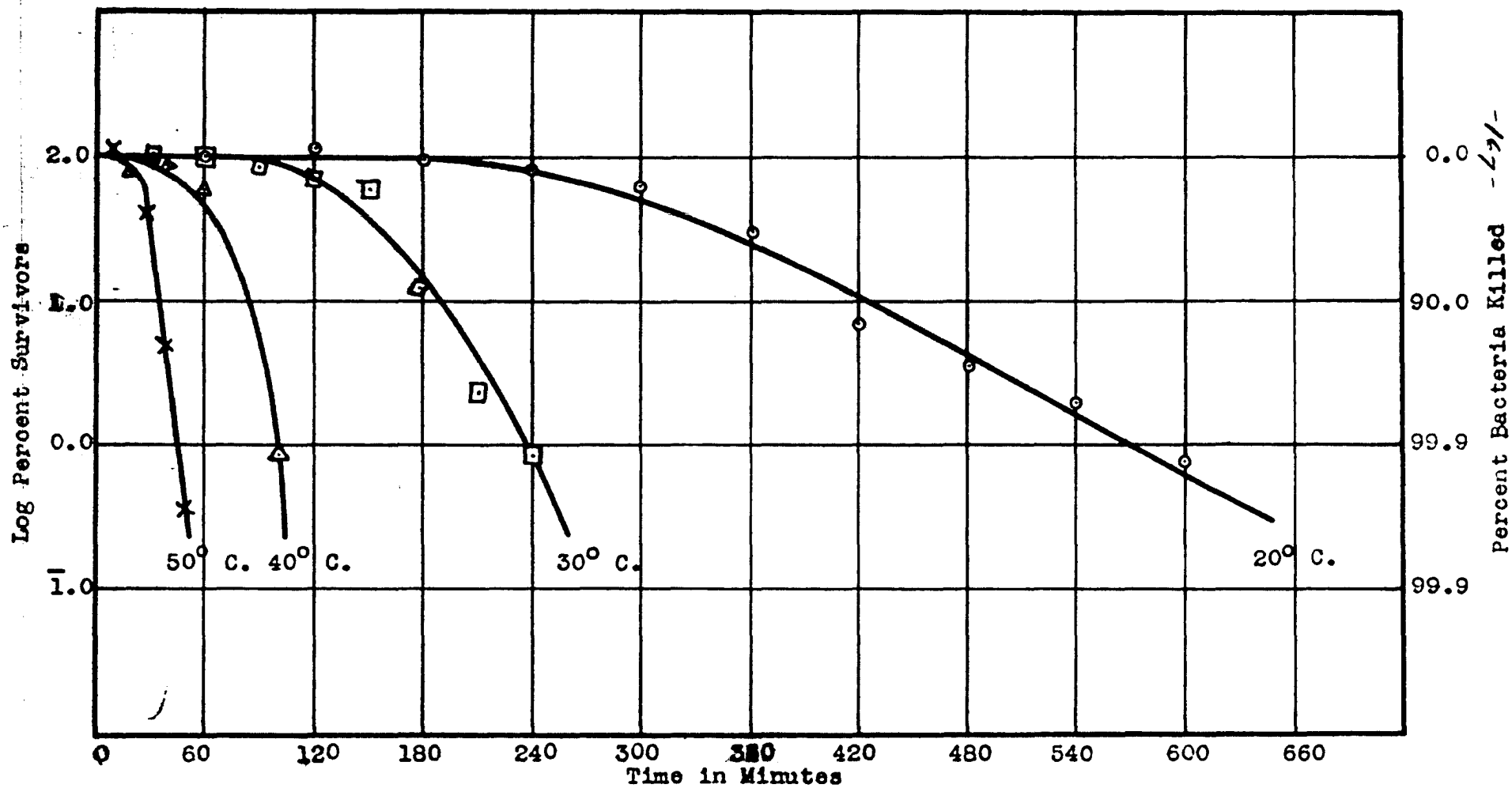


TABLE 10A

INFLUENCE OF TEMPERATURE ON RESISTANCE OF B. METIENS SPORES TO CHLORINE

(25 p.p.m. Av. Cl. added; pH 10.0)

Temp. Degrees C.	Residual Av. Cl. after 15 min. (initial)	% of Av. Cl. added remaining after 15 min.	Time (min.) to kill 99%	Log time (min.) to kill 99%	Log Temp. degrees C.	Ratio times (min.) to kill 99%
20#	24.2	97	570	2.76	1.30	-----
30	23.3	93	240	2.38	1.48	$\frac{570}{240} = 2.4$
40	23.2	93	100	2.00	1.60	$\frac{240}{100} = 2.4$
50	22.8	91	46	1.66	1.70	$\frac{100}{46} = 2.2$

See Table 6 and Fig. 6.

Fig. 10A

INFLUENCE OF TEMPERATURE ON RESISTANCE OF B. [illegible] SPORES TO CHLORINE SOLUTION
(25 p.p.m. Av. Cl. added; pH 10.0)

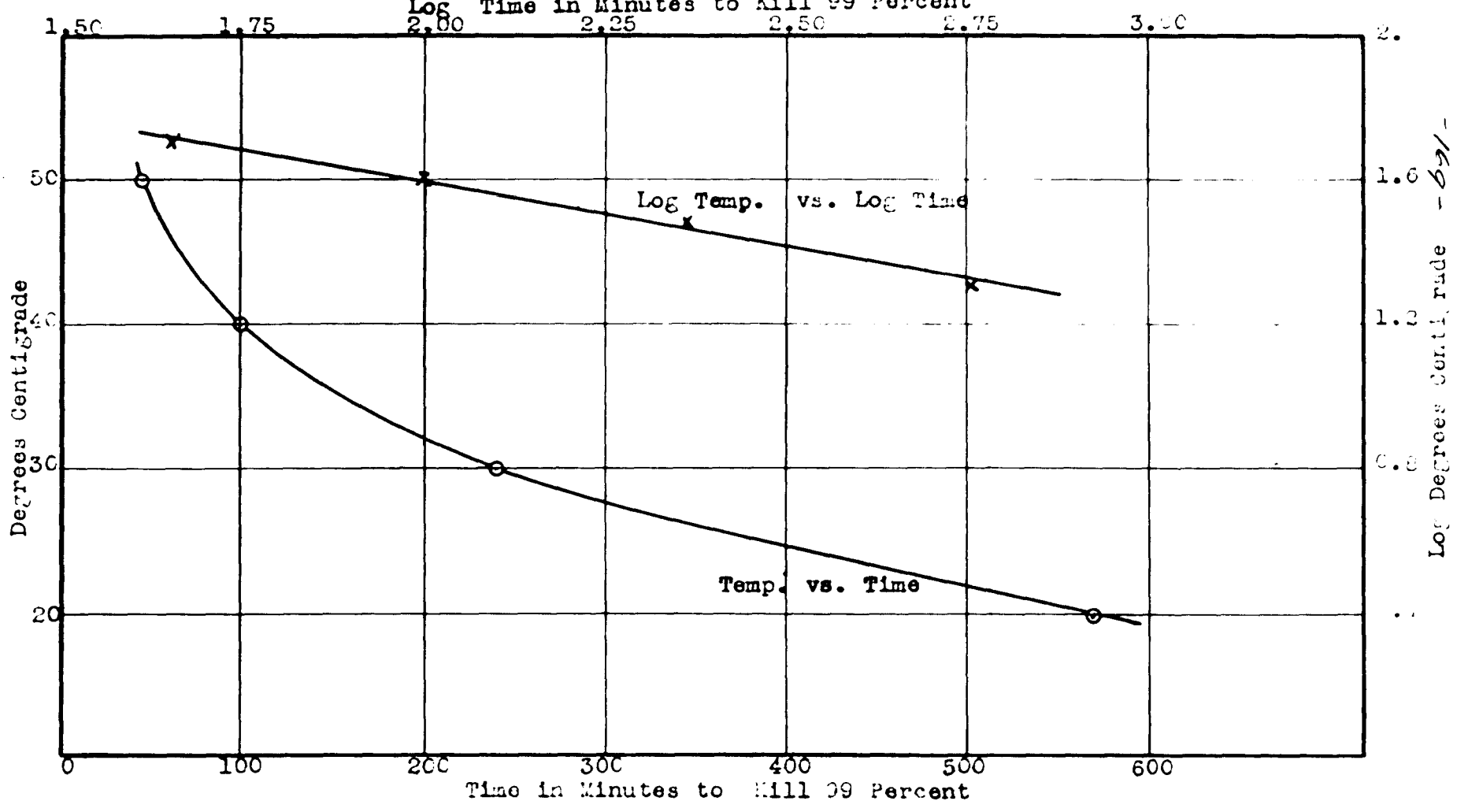


TABLE 10B

SUMMARY OF DATA SHOWING EFFECT OF TEMPERATURE ON RESISTANCE OF *B. METIENS* SPORES TO CHLORINE SOLUTION

(25 p.p.m. Av. Cl. added; pH 10.0; see Tables 10 and 10A)

Temperature degrees C.	20	30	40	50
Killing time* (min.)	570	240	100	46
Residual Av. Cl. (p.p.m.) after 15 min. contact #	24.2	23.3	23.2	22.8
Residual Av. Cl. (p.p.m.) at end of experiment	20.3	20.5	19.8	19.3
pH at end of experiment	9.98	9.98	9.98	9.98

* Time to kill 99% of exposed spores.

Residual Av. Cl. after 15 minutes contact of chlorine with the buffered solution.

5. Effect of temperature on the germicidal efficiency of chlorine-ammonia solutions at pH 10.

To 25 p.p.m. available chlorine were added 6 p.p.m. of ammonia and the germicidal efficiency of the mixture ascertained at 20° C., 30° C., 40° C., and 50° C. Results are shown in Table 11 and survivor curves are presented in Figure 11. The influence of temperature on the germicidal efficiency of chlorine-ammonia solutions is shown in Table 11A and Figure 11A. Table 11B shows the initial chlorine concentrations at the time that the test organism was added when 25 p.p.m. available chlorine and 6 p.p.m. ammonia were added to the buffered solution at pH 10. The initial chlorine concentration was not significantly different at the various temperatures.

At 20° C., the initial chlorine concentration was 23.0 p.p.m. and the residual at the end of the experiment was 20.6 p.p.m. The time required to kill 99 percent of the exposed spores was 186 minutes.

When the temperature was raised to 30° C. the initial chlorine was 21.7 p.p.m. and remained constant for the duration of the experiment with a killing time of 46 minutes.

At 40° C. the initial chlorine was 22.3 p.p.m. with a drop in residual to 20.5 p.p.m. at the end of the experiment. The killing time was 10.4 minutes.

Increasing the temperature to 50° C. resulted in an initial concentration of 20.7 p.p.m. with no appreciable change during the course of the experiment. The time required to kill 99 percent of the exposed spores was only 3.3 minutes.

The reaction (pH) determined at the end of the experiments was constant varying only from pH 9.98 to 9.99.

Figure 11A shows that when the logarithm of the killing time is plotted against the logarithm of the temperature the points fall on a straight line the equation for which is

$$\log y = (-0.222) \log x + 1.833$$

where y is the temperature in degrees C. and x is the killing time in minutes. It may be seen that a rise in temperature of 10° C. results in a decrease in killing time of approximately 75 percent.

TABLE 11

EFFECT OF TEMPERATURE ON RESISTANCE OF B. METIENS SPORES TO CHLORINE-AMMONIA SOLUTION
(25 p.p.m. Av. Cl. and 6 p.p.m. NH_3 added; pH 10.0)

Temperature Degrees C.	20			30				
Expt. No.	*			Expt. No.	164	166		
Date				Date	3/22/40	3/28/48		
Exposure Time (in min.)		Av. % Survivors*	Log. Av. % Survivors*	Exposure Time (in min.)	** Survivors	** Survivors	Av. % Survivors	Log. Av. % Survivors
0		100	2.00	0	800	1,600	100	2.00
30		53	1.72	10	---	850	---	----
60		23	1.36	20	165	345	22	1.34
90		12	1.08	30	---	42.5	---	----
120		5.4	0.73	40	32.5	22.5	2.8	0.45
150		1.9	0.29	50	---	11	---	----
180		1.0	0.00	60	3.25	3.25	0.30	T.48
210		0.48	T.68					
240		-----	----					
270		-----	----					

Temperature Degrees C.	40				50				
Expt. No.	162	169			Expt. No.	160	168		
Date	3/21/40	3/28/40			Date	3/21/40	3/28/40		
Exposure Time (in min.)	** Survivors	** Survivors	% Survivors#	Log % Survivors	Exposure Time (in min.)	** Survivors	** Survivors	Av. % Survivors	Log. Av. % Survivors
0	1,450	650	100	2.00	0.0	1,850	1,350	100	2.00
2	-----	425	65	1.82	0.5	900	650	49	1.69
4	-----	205	32	1.50	1.0	550	285	26	1.42
6	-----	90	12	1.09	1.5	345	110	14	1.15
8	-----	15	2.3	0.36	2.0	150	60	6.3	0.80
10	39.5	9	1.4	0.14	2.5	90	29.5	3.6	0.56

180		1.0	0.00						
210		0.48	I.68						
240		-----	----						
270		-----	----						

Temperature Degrees C.	40				50				
Expt. No.	162	169			Expt. No.	160	168		
Date	3/21/40	3/28/40	%	Log %	Date	3/21/40	3/28/40	Av. %	Log. Av. %
Exposure Time (in min.)	** Survivors	** Survivors	Survivors#	Survivors	Exposure Time (in min.)	** Survivors	** Survivors	Survivors	Survivors
0	1,450	650	100	2.00	0.0	1,850	1,350	100	2.00
2	-----	425	65	1.82	0.5	900	650	49	1.69
4	-----	205	32	1.50	1.0	550	285	26	1.42
6	-----	90	12	1.09	1.5	345	110	14	1.15
8	-----	15	2.3	0.36	2.0	150	60	6.3	0.80
10	39.5	9	1.4	0.14	2.5	90	29.5	3.6	0.56
12	-----	4.75	0.73	I.86	3.0	26	19.5	1.4	0.15
20	0.75	---	---	----	3.5	13	13	0.83	I.94
					4.0	3	5	0.27	I.43

Temperature Degrees C.	20	30			40			50		
Expt. No. *	Average	164	166	Average	162	169	Average	160	168	Average
Av. Cl. Residual at end of Experiment	20.6	21.3	22.0	21.7	20.6	20.5	20.6	20.3	20.6	20.5
pH at end of Experiment	9.99	9.98	9.98	9.98	9.98	9.99	9.99	9.97	9.99	9.98
Killing time (min.) ***	186	48	44	46	##	10.4	-----	3.3	3.3	3.3

* See Table 6C
** Surviving bacteria (in thousands) per 5 ml.
*** Time for killing 99%
\# For Expt. No. 169 only
Not determined

Fig. 11

EFFECT OF TEMPERATURE ON RESISTANCE OF B. METIENS SPORES TO CHLORINE-AMMONIA SOLUTION
(25 p.p.m. Av. Cl. and 6 p.p.m. NH_3 added; pH 10.0)

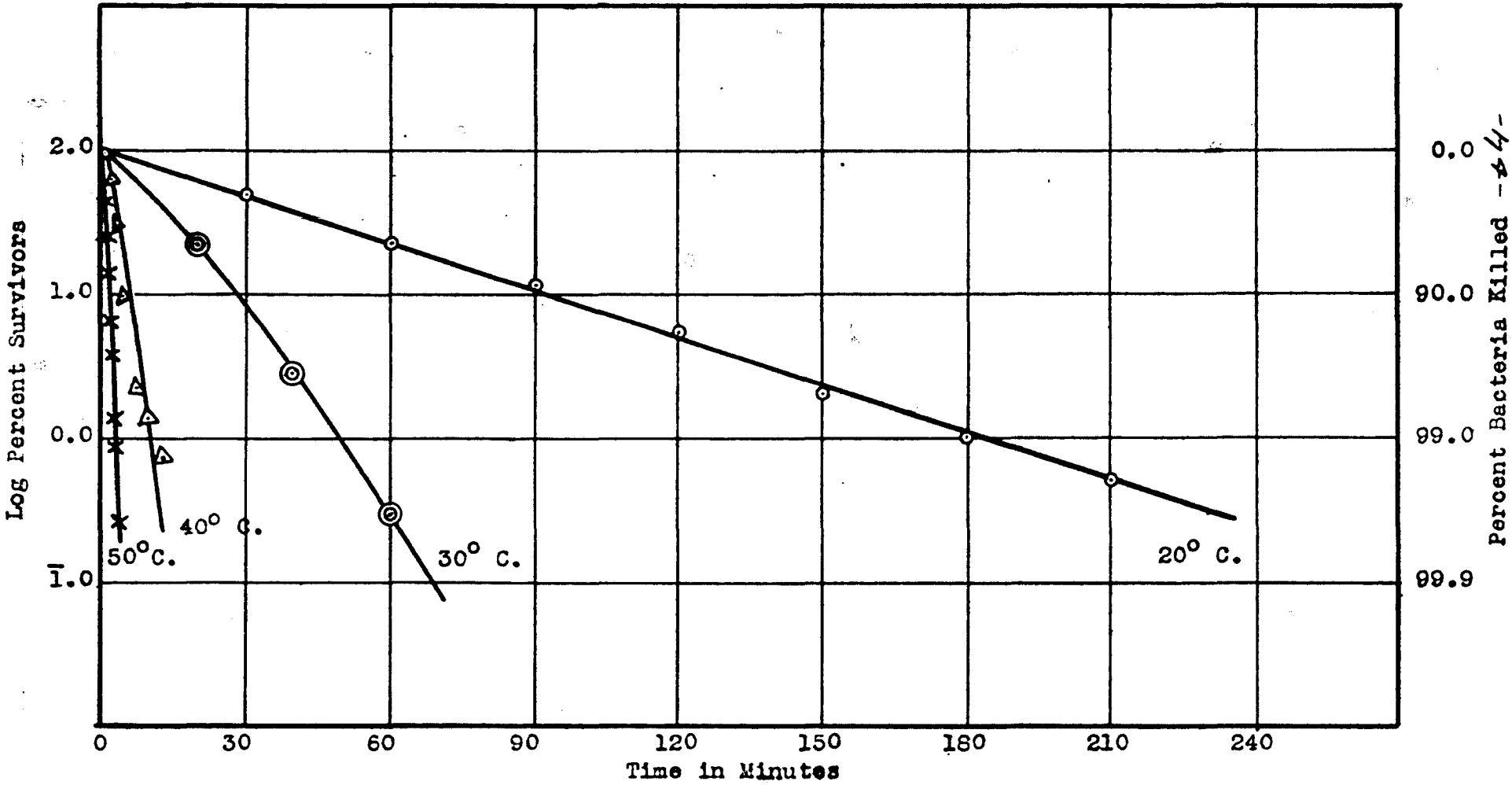


TABLE 11A

INFLUENCE OF TEMPERATURE ON RESISTANCE OF B. METIENS SPORES TO CHLORINE-AMMONIA SOLUTION

(25 p.p.m. Av. Cl. and 6 p.p.m. NH_3 added; pH 10.0)

Temp. Degrees C.	Residual Av. Cl. after 15 min. (initial	% of Av. Cl. added remaining after 15 min.	Time (min.) to kill 99%	Log Time (min.) to kill 99%	Log Temp. degrees C.	Ratio times (min.) to kill 99%
20#	23.0	92	186	2.27	1.30	-----
30	21.7	87	46	1.66	1.48	$\frac{186}{44} = 4.2$
40	22.3	89	10.4	1.02	1.60	$\frac{44}{10.4} = 4.2$
50	20.7	83	3.3	0.52	1.70	$\frac{10.4}{3.3} = 3.3$

See Table 6C and Fig. 6C.

Fig. 11A

INFLUENCE OF TEMPERATURE ON RESISTANCE OF B. METIENS SPORES TO CHLORINE-AMMONIA SOLUTION

(25 p.p.m. Av. Cl. and 6 p.p.m. added; pH 10.0)

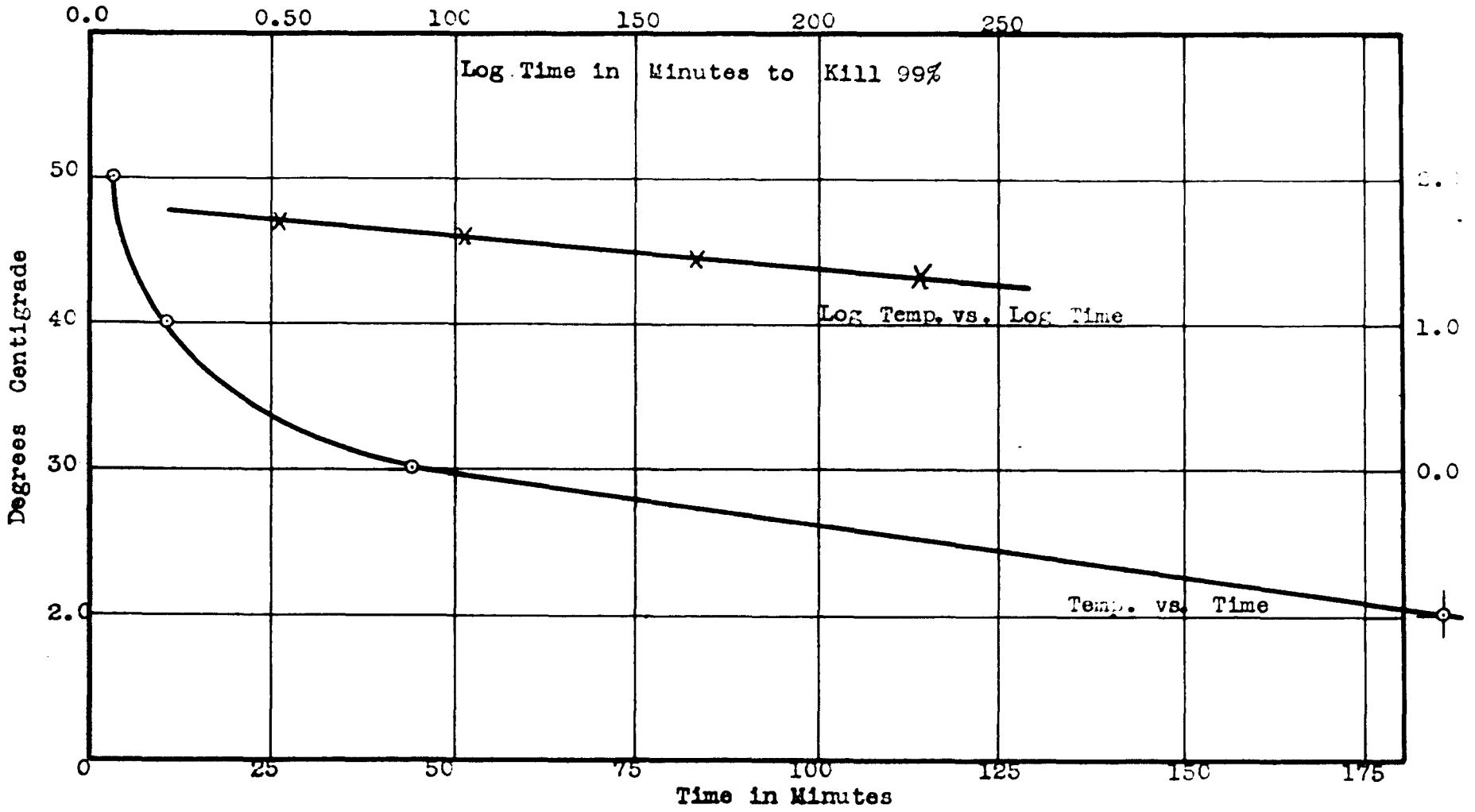


TABLE 11B

SUMMARY OF DATA SHOWING EFFECT OF TEMPERATURE ON RESISTANCE OF B. METIENS SPORES TO CHLORINE-AMMONIA SOLUTION

(25 p.p.m. Av. Cl. and 6 p.p.m. NH_3 added; pH 10.0; See Tables 11 and 11A)

Temperature degrees C.	20	30	40	50
Killing time* (min.)	186	46	10.4	3.3
Residual Av. Cl. (p.p.m.) after 15 min. contact#	23.0	21.7	22.3	20.7
Residual Av. Cl. (p.p.m.) at end of experiment	20.6	21.7	20.5	20.5
pH at end of experiment	9.99	9.98	9.99	9.98

* Time to kill 99% of exposed spores.

Residual Av. Cl. after 15 minutes contact of chlorine with the buffered solution.

Figure 11B is a summary of Figures 10A and 11A, showing the relative effect of temperature on the resistance of B. metiens spores to chlorine and chlorine-ammonia solutions. The killing times are shown in Table 11C. In the upper right-hand quadrant of Figure 11B are graphs for chlorine and chlorine-ammonia solutions in which the logarithm of the killing time has been plotted against the logarithm of the temperature. It will be noted that the graphs for the chlorine and the chlorine-ammonia solutions are straight, the slopes of which are -0.370 and -0.222 respectively. It appears that if these lines were extended far enough they would intercept, and at the temperature represented by that point (approximately 10° C.), the same killing time would be obtained for both the chlorine and the chlorine-ammonia solutions.

TABLE 11C

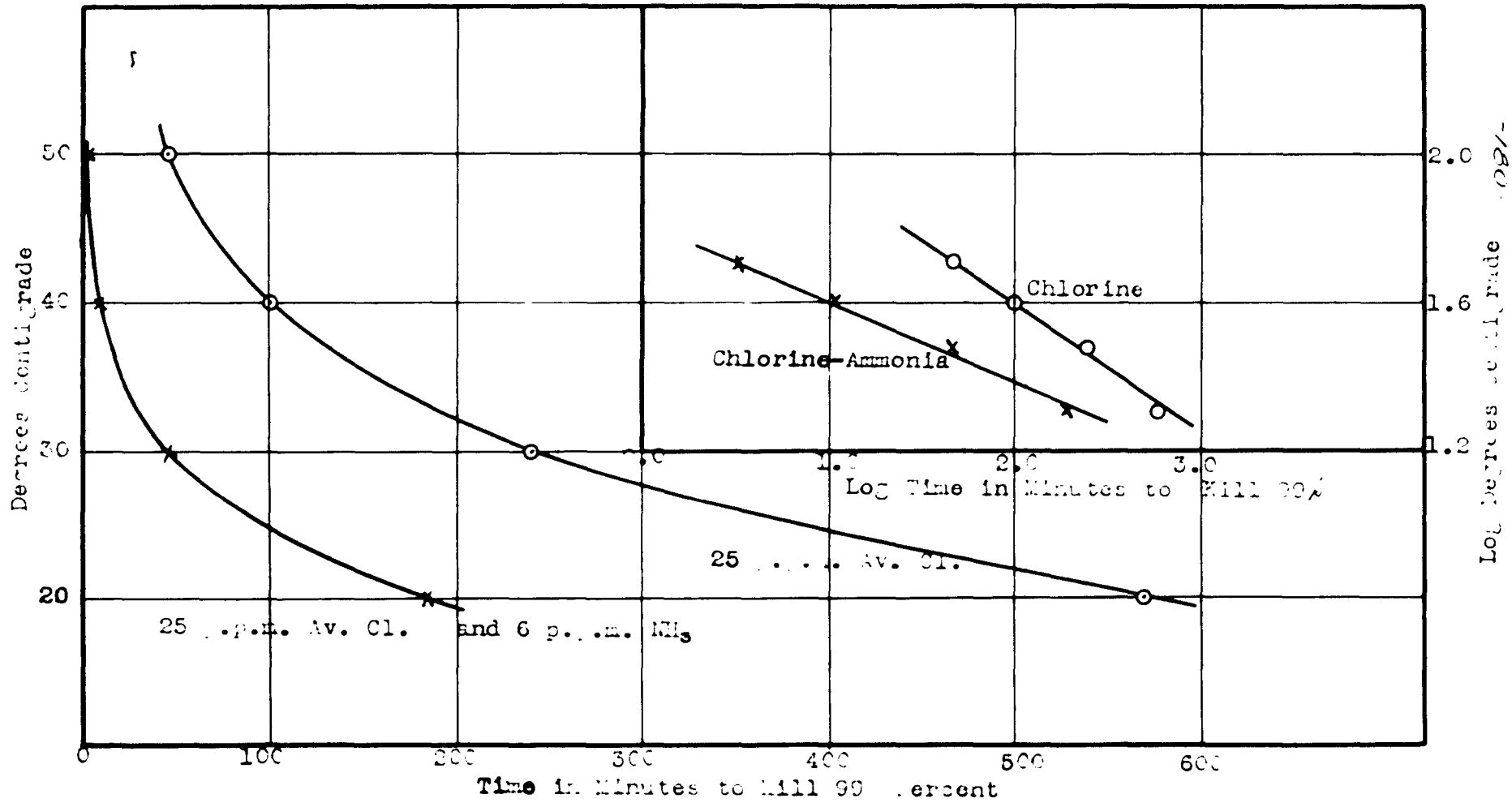
SHOWING INFLUENCE OF TEMPERATURE ON RESISTANCE OF B. METIENS SPORES TO CHLORINE AND CHLORINE-AMMONIA SOLUTIONS (pH 10.0; See Tables 10A and 11A)

Temperature (degrees C.)	Time to Kill 99% Exposed Spores (min.)	
	25 p.p.m. Av. Cl.	25 p.p.m. Av. Cl. and 6 p.p.m. NH_3
20	570	186
30	240	46
40	100	10.4
50	46	3.3

Fig. 11B

SHOWING IN LOGARITHMIC TERMS THE RELATIVE RESISTANCE OF B. METIENS SPORES TO CHLORINE AND CHLORINE-AMMONIA SOLUTION

(T 10.6; see Fig. 10B and Fig. 11A)



IV. SUMMARY AND CONCLUSIONS

1. A technique has been developed for maintaining constant reactions (pH) of germicidal test solutions of chlorine and chloramines.

2. Bacterial spores were considered particularly suitable for this study since by using resistant (spore-forming) organisms instead of vegetative cells it was possible to study wider ranges of reaction (pH), temperature, and concentration. The test organism used (Bacillus metiens) has previously been employed in studies on disinfection with alkalies, chloramine-T, and calcium hypochlorite.

3. Spore suspensions, prepared in Butterfield's formula "C" water and stored at 10° C. showed no appreciable change in resistance to buffered chlorine solutions for a period of more than nine months. This confirms results reported by Rudolph and Levine (1938) on the resistance of B. metiens spores against calcium hypochlorite solutions.

4. The influence of reaction (pH) on the germicidal efficiency of chlorine and chloramines was observed by determining the time required to kill 99 percent of B. metiens spores at pH 5, 6, 7, 8, 9 and 10 in buffered solutions with the following results:

a. With initial concentrations of chlorine at 22 to 24 p.p.m. as hypochlorites, the killing times were very short in the range pH 5 to pH 7, namely, 2.1 to 3.0 minutes. At pH 8 the killing time rose to 7.6 minutes, at pH 9 to 58 minutes, and at pH 10 it increased very markedly to 570 minutes.

b. When 0.5 and 2 p.p.m. ammonia were added to 25 p.p.m. available chlorine, in buffered solutions at 20° C., there was a drop in chlorine concentration corresponding to approximately 7 times the ammonia added, due to the oxidation of ammonia, the residual chlorine existing as hypochlorite. Except for the fact that the killing times were increased because of the lower residual chlorine, the effect of reaction was identical with that reported above for hypochlorite.

c. The effect of reaction on the germicidal efficiency of chloramines (about 25 p.p.m. available chlorine) was in marked contrast to that observed for hypochlorites. Thus, when 6 p.p.m. of ammonia was added to 25 p.p.m. chlorine, (the theoretical ratio of chlorine to ammonia for chloramine) killing times of 83 to 89 minutes were obtained at pH 8 to 6 at a more acid reaction, pH 5, the killing time rose to 168 minutes and, similarly, at more alkaline reactions, pH 9 and 10, the killing times had risen to 182 and 186 minutes, respectively.

On addition of more ammonia (18 p.p.m.) the killing times for the more acid solutions were reduced whereas those for the alkaline solutions were distinctly increased over what was observed with the mixture consisting of 6 p.p.m. ammonia and 25 p.p.m. available chlorine. Thus a minimum killing time of 59 minutes was observed at pH 6 but this rose to 99 minutes in the more acid solution, pH 5, whereas at pH 7 a killing time of 84 minutes was obtained and at more alkaline solutions, pH 8, 9, and 10, the killing times rose very rapidly to 107, 263 and 456 minutes, respectively. It appears, therefore, that there is an optimum reaction for germicidal efficiency of chlorine-ammonia mixtures, not far from the neutral point, when the killing time is at a minimum, whereas, with hypochlorite solutions, the killing time decreases as the solution becomes more acid.

d. Chloramine was found to be more efficient as a germicide than hypochlorite in alkaline solutions but the hypochlorites were markedly more efficient than chloramine (25 p.p.m. chlorine and 6 p.p.m. ammonia) in acid solutions. Plots of the killing times for approximately 25 p.p.m. available chlorine as chloramine and as hypochlorite against reaction, cross at approximately pH 9.3. At the more alkaline reaction of pH 10, chloramine showed a killing time of 186 minutes as compared with 570 minutes for the hypochlorite; at a more acid reaction, as for example pH 8, killing times for chloramine and hypochlorite were 83 minutes and 7.6 minutes,

respectively.

e. The curve for chloramine (6 p.p.m. ammonia) crossed the curve for chloramine with excess ammonia (18 p.p.m.) at about pH 7.25. Thus, the addition of ammonia to chloramine resulted in little change in killing time near neutrality, a shorter killing time at acid reactions (pH 6 and 5) and an increased killing time at alkaline reactions (pH 8, 9 and 10).

5. The effect of concentration on the germicidal efficiency of chlorine (hypochlorite) and chloramine (ratio of available chlorine to ammonia = 4.2/1) was observed in buffered solutions at pH 10 and 20° C. with the following results:

a. When the logarithms of the initial concentrations of chlorine as hypochlorite were plotted against the logarithms of the killing times, the points fell on a straight line. The equation for this line is

$$\log y = (-0.860) \log x + 3.936$$

where y is the killing time in minutes and x is the initial concentration of available chlorine in p.p.m. In general, it may be said that when the chlorine concentration was doubled (between 25 and 200 p.p.m. available chlorine) the killing time was reduced by about 60 percent.

b. Plotting the logarithms of the initial available chlorine concentrations (as chloramine) against the logarithms

of the killing times gave a slightly curved line. As the concentration of chloramine (expressed as available chlorine) increased, the relative decrease in killing time progressively became less.

6. The effect of temperature on the germicidal efficiency of chlorine (hypochlorite) and the chloramine (25 p.p.m. available chlorine and 6 p.p.m. ammonia) was determined by observing the killing times of approximately 25 p.p.m. available chlorine at pH 10 for temperatures of 20° C., 30° C., 40° C., and 50° C.

a. For hypochlorite it was found that a plot of the logarithms of the killing times against the logarithms of the temperatures approximated a straight line, the equation for which is

$$\log y = (-0.370) \log x + 2.334$$

where y is the temperature in degrees centigrade and x is the killing time in minutes. For each increase in the temperature of 10° C. the killing time was shortened by about 60 percent.

b. For chloramine it was observed that when the logarithms of the killing times were plotted against the logarithms of the temperatures, the points fell on a straight line. The equation for this line is

$$\log y = (-0.222) \log x + 1.833$$

where y is the temperature in degrees centigrade and x is the killing time in minutes. A rise of 10° C. resulted in a decrease in the killing time of approximately 75 percent.

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VII. APPENDIX

Directions for the preparation of M/5 buffers used in this study are given below. All water used was freshly boiled (distilled) and cooled so as to expel dissolved carbon dioxide. The sodium hydroxide solution was likewise prepared so as to contain a minimum amount of carbonate. All reactions are stated for M/20 buffer solutions, using one part M/5 buffer and three parts water.

pH 5.0 (M/5)#

6.8045 grams $NaC_2H_3O_2$

16.00 ml. N/1 H_2SO_4

make up to 250 ml. with water.

pH 6.0 (M/5)*

6.8075 grams KH_2PO_4

5.64 ml. N/1 NaOH

make up to 250 ml. with water.

pH 7.0 (M/5)*

6.8075 KH_2PO_4

29.54 ml. N/1 NaOH

make up to 250 ml. with water.

See following page for footnotes.

pH 8.0 (M/5)*

6.8075 grams KH_2PO_4

46.85 ml. N/1 NaOH

make up to 250 ml. with water

pH 9.0 (M/5)#

4.2005 grams $NaHCO_3$

5.0 ml. N/1 NaOH

make up to 250 ml. with water

pH 10.0 (M/5) #

Solutions were prepared as follows:

(1) M/5 Na_2CO_3

5.2995 grams Na_2CO_3, made up to 250 ml. with water.

(2) M/5 $NaHCO_3$

4.2005 grams $NaHCO_3$, made up to 250 ml. with water.

(3) Mix 250 ml. of M/5 Na_2CO_3 with 200 ml. of M/5 $NaHCO_3$.

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* Solutions were sterilized in the autoclave.

Solutions were not sterilized in the autoclave since the high temperature caused a change in reaction (pH). Salts were dissolved in sterile water using sterile equipment and stored in a refrigerator at 10° C. Plate counts showed that the solutions were sterile.